MR 58819

# VINYL ACETATE TOXICOLOGY GROUP, INC.

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8EHQ-0502-15146

April 23, 2002




VIA FEDERAL EXPRESS
U.S. Environmental Protection Agency
ATTN: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
1201 Constitution Avenue, NW
Room 3166
Washington, DC 20460

**Re: TSCA 8(e) Submission: Japan Bioassay Research Center Report**

Dear TSCA 8(e) Coordinator:

On behalf of the Vinyl Acetate Toxicology Group (VATG), I am submitting supplemental information relating to a two-year cancer bioassay on vinyl acetate conducted by the Japan Bioassay Research Center (JBRC). The VATG represents the major North American producers and processors of vinyl acetate. Members of the VATG include: AT Plastics, Inc.; Air Products & Chemicals, Inc.; Borden, Inc.; Celanese Limited; Dow Chemical Company; E. I. Du Pont de Nemours and Company; ExxonMobil Biomedical Sciences, Inc.; and, Millennium Petrochemicals.


The study report is dated November 1995 and was initiated over 10 years ago. The study was conducted at the Japan Bioassay Research Center of the Japan Industrial Safety and Health Association (JISHA) which is located at 2445 Hirasawa Hadano Kanagawa, 257-0015 Japan. Preliminary information on the results of this study was made available to the EPA in 1997 and placed in the U.S. EPA/OPTS public files (see Fiche No. OTS0001286, Doc. No. FYI-OTS-0297-1286).

Enclosed you will find two separate reports. The first is a more complete version of the 1997 submission noted above and is the original report received, which is largely in Japanese (note that we do not have a copy of pages 4-5). The second file is an English translation of the report as prepared by a translation service based in Arlington, Virginia. The VATG has identified several technical issues regarding this study and expects to discuss its findings with the relevant EPA officials as part of the Agency's ongoing update of the vinyl acetate IRIS file. We cannot confirm the accuracy of the translation but believe it to be reliable.



Please do not hesitate to contact me (202.637.9040 or bobf@regnet.com) if I can provide further clarification.

Sincerely,

Robert J. Fensterheim
Executive Director

Contain NO CBI

MR58819



# Report on Carcinogenicity Studies on Vinyl Acetate Orally Administered (mixed in drinking water) to Rats and Mice



**November 30, 1995**

**(Reduced Print Edition)**

**Japan Bioassay Research Center**
**Japan Industrial Safety and Health Association (JISHA)**

[Copy of Japanese-to-English Translation by Comprehensive Language Center, Inc., Arlington, VA]

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**Abstract**

Two-year (104-week) studies in which vinyl acetate was orally given (mixed in drinking water) to rats and mice were conducted in order to determine the carcinogenicity of the substance.

The animals used in the studies were F344/DuCrj (Fisher) rats and CRJ:$BDF_1$ mice. With 50 males and 50 females each allocated to a group, three groups received the test chemical, and 1 group was used as the control group for a total of four groups. A total of 400 rats and 400 mice were used.

The animals were administered the vinyl acetate by allowing them to freely consume drinking water into which had been mixed vinyl acetate at the concentration associated with their respective group. The concentrations were 400 ppm, 2,000 ppm, and 10,000 ppm (geometric ratio of 5.0) for both male and female rats and mice. The general condition of the animals was observed, their body weight, amount of water consumed, and amount of food consumed were measured, and the following tests were performed: hematological tests, blood biochemistry tests, urinalysis, necropsy, organ weight measurements, and histopathologic tests.

In the male rats, squamous cell carcinomas and squamous cell papillomas were identified in the oral cavity of males in the 10,000 ppm group, and in the female rats, squamous cell carcinoma occurred in the oral cavity in the 400 ppm group and above and in the esophagus of the 10,000 ppm group.

Lesions thought to be the early stages of tumors were also identified. In both males and females of the 10,000 ppm group, squamous cell hyperplasia, activation of the basal cells, and dysplasia of the epithelium were noted in the oral cavity, esophagus, and stomach.

In both the male and female mice of the 10,000 ppm group, squamous cell carcinomas and squamous cell papillomas were noted in the oral cavity and stomach, and squamous cell carcinoma occurred in the esophagus and larynx. In addition, squamous cell papilloma of the esophagus and squamous cell carcinoma of the larynx was uncommon but present in the females of the 2,000 ppm group. Squamous cell carcinoma of the larynx near the oral cavity was noted in males of the 10,000 ppm group and females of the 2,000 and 10,000 ppm groups. Lesions that appeared to be the early stages of cancer included squamous epithelia hyperplasia, basal cell activation, and epithelial dysplasia in the oral cavity, esophagus, stomach and the squamous epithelium, or the covering epithelium, of the larynx in both males and females of the 10,000 ppm group. Although uncommon, epithelial hyperplasia, basal cell activation, and dysplasia were noted in the epithelium of the oral cavity in the 2,000 ppm group.

This study, in which vinyl acetate was administered orally for 2 years in drinking water in the above manner, verified the carcinogenicity of vinyl acetate, indicating an increase in the incidence of oral squamous cell carcinoma and squamous cell papilloma in male F334/DuCrj (Fisher) rats and in squamous cell carcinoma of the mouth and esophagus in their female counterparts.

And in male and female CRJ:$BDF_1$ mice, an increase in the incidence of squamous cell carcinoma and squamous cell papilloma in the oral cavity and stomach and squamous cell carcinoma of the esophagus and larynx were observed, thus verifying the carcinogenicity of vinyl acetate.

Incidence of Major Tumors in Vinyl Acetate Carcinogenicity Study (Male Rats)

| | Concentration administered (ppm) | | 0 | 400 | 2,000 | 10,000 | Peto test | Cochran-Armitage test |
|---|---|---|---|---|---|---|---|---|
| | Number of animals tested | | 50 | 50 | 50 | 50 | | |
| Benign tumors | Skin | Acanthoma | 3 | 2 | 4 | 1 | | |
| | Subdermal tissue | Fibroma | 3 | 4 | 8 | 3 | | |
| | Lungs | Bronchiolar/alveolar adenoma | 3 | 1 | 3 | 1 | | |
| | Oral cavity | Squamous cell papilloma | 0 | 0 | 0 | 2 | | |
| | Pituitary gl. | Adenoma | 19 | 16 | 14 | 11 | | |
| | Thyroid gl. | C-cell adenoma | 7 | 9 a) | 3 a) | 7 a) | | |
| | Islet cells | Islet cell adenoma | 6 | 5 | 4 | 3 | | |
| | Adrenals | Melanocytoma | 8 | 4 | 9 | 7 | | |
| | Testes | Interstitial cell carcinoma | 42 | 40 | 44 | 47 | | |
| Malignant tumors | Spleen | Mononuclear leukemia | 3 | 5 | 7 a) | 4 | | |
| | Oral cavity | Squamous cell carcinoma | 0 | 0 | 0 | 5* | ↑↑ | ↑ |

Incidence of Major Tumors in Vinyl Acetate Carcinogenicity Study (Female Rats)

| | Concentration administered (ppm) | | 0 | 400 | 2,000 | 10,000 | Peto test | Cochran-Armitage test |
|---|---|---|---|---|---|---|---|---|
| | Number of animals tested | | 50 | 50 | 50 | 50 | | |
| Benign tumors | Pituitary gl. | Adenoma | 8 | 11 | 9 | 14 | | |
| | Thyroid gl. | C-cell adenoma | 2 | 7 | 8 | 5 | | |
| | Islet cells | Islet cell adenoma | 0 | 1 | 3 | 1 | | |
| | Adrenals | Melanocytoma | 4 | 1 | 1 | 2 | | |
| | Uterus | Intrauterine intermemb. polyp | 5 | 5 | 10 | 4 | | |
| | Mammary glands | Adenoma | 1 | 3 | 0 | 1 | | |
| | | Fibroadenoma | 9 | 10 | 8 | 9 | | |
| | Clitoral gland | Adenoma | 2 | 0 | 3 | 1 | | |
| Malignant tumors | Spleen | Mononuclear leukemia | 4 | 5 | 8 | 7 | | |
| | Oral cavity | Squ. cell carcinoma | 0 | 1 | 1 | 3 | ↑ | |
| | Esophagus | Squ. cell carcinoma | 0 | 0 | 0 | 1 | | |
| | Mammary glands | Adenocarcinoma | 0 | 0 | 0 | 3 | | |

The results are presented in consideration of biological significance.

* Significant at significance level of 5% or less

** Significant at significance level of 1% or less (Fisher's exact test)

↑: Significance increases at significance level of 5% or less

↑↑: Significance increases at significance level of 1% or less (Peto/Cochran-Armitage tests)

↓: Significance decreases at significance level of 5% or less

↓↓: Significance decreases at significance level of 1% or less (Cochran-Armitage test)

a) 49 animals were tested. The number of animals tested elsewhere is as specified.

## Incidence of Major Tumors in Vinyl Acetate Carcinogenicity Study (Male Mice)

| | Concentration administered (ppm) | | 0 | 400 | 2,000 | 10,000 | Peto test | Cochran-Armitage test |
|---|---|---|---|---|---|---|---|---|
| | Number of animals tested | | 50 | 50 | 50 | 50 | | |
| Benign tumors | Lungs | Bronchiolar/alveolar adenoma | 3 | 3 | 4 | 3 | | |
| | Oral cavity | Squ. cell papilloma | 0 | 0 | 0 | 4 | ↑↑ | ↑↑ |
| | Stomach | Squ. cell papilloma | 0 | 0 | 0 | 2 | | |
| | Liver | Hepat. adenoma | 2 | 6 | 4 | 3 | | |
| | Harderian gl. | Adenoma | 3 | 4 | 6 | 2 | | |
| | All organs | Angioma | 3 | 0 | 2 | 0 | | |
| Malignant tumors | Larynx | Squ. cell carcinoma | 0 | 0 | 0 | 2 | | |
| | Lungs | Bronchiolar/alveolar adenoma | 7 | 3 | 5 | 2 | | |
| | Lymph nodes | Malig. lymphoma | 5 | 6 | 6 | 3 | | |
| | Spleen | Angiosarcoma | 2 | 1 | 4 | 0 | | |
| | Oral cavity | Squ. cell carcinoma | 0 | 0 | 0 | 13** | ↑↑ | ↑↑ |
| | Esophagus | Squ. cell carcinoma | 0 | 0 | 0 | 7* | ↑↑ | ↑↑ |
| | Stomach | Squ. cell carcinoma | 1 | 0 | 0 | 7* | ↑↑ | ↑↑ |
| | Liver | Angiosarcoma | 4 | 5 | 5 | 4 | | |
| | | Hepat. carcinoma | 13 | 10 | 9 | 4* | | ↓ |
| | All organs | Malig. lymphoma | 6 | 7 | 8 | 3 | | |
| | | Angiosarcoma | 6 | 7 | 7 | 4 | | |

## Incidence of Major Tumors in Vinyl Acetate Carcinogenicity Study (Female Mice)

| | Concentration administered (ppm) | | 0 | 400 | 2,000 | 10,000 | Peto test | Cochran-Armitage test |
|---|---|---|---|---|---|---|---|---|
| | Number of animals tested | | 50 | 50 | 50 | 50 | | |
| Benign tumors | Lungs | Bronchiolar/alveolar adenoma | 1 | 3 | 1 | 2 | | |
| | Oral cavity | Squ. cell papilloma | 0 | 0 | 0 | 3 | ↑↑ | ↑↑ |
| | Esophagus | Squ. cell papilloma | 0 | 0 | 1 | 0 | | |
| | Stomach | Squ. cell papilloma | 0 | 0 | 0 | 1 | | |
| | Liver | Hepat. adenoma | 3 | 1 | 4 | 0 | | |
| | Pituitary gl. | Adenoma | 10 | 6 [a)] | 8 [a)] | 7 | | |
| | Harderian gl. | Adenoma | 0 | 4 | 3 | 0 | | |
| Malignant tumors | Larynx | Squ. cell carcinoma | 0 | 0 | 1 | 1 | | |
| | Lungs | Bronchiolar/alveolar adenoma | 2 | 3 | 1 | 1 | | |
| | Lymph nodes | Malig. lymphoma | 11 | 10 | 17 | 10 | | |
| | Spleen | Malig. lymphoma | 0 | 5* | 1 | 1 | | |
| | Oral cavity | Squ. cell carcinoma | 0 | 0 | 0 | 15** | ↑↑ | ↑↑ |
| | Esophagus | Squ. cell carcinoma | 0 | 0 | 0 | 1 | | |
| | Stomach | Squ. cell carcinoma | 0 | 0 | 0 | 3 | ↑↑ | ↑↑x |
| | Liver | Angiosarcoma | 3 | 3 | 0 | 1 | | |
| | Uterus | Histiosarcoma | 10 | 11 | 8 | 10 [b)] | | |
| | All organs | Malig. lymphoma | 11 | 16 | 18 | 11 | | |
| | | Angiosarcoma | 4 | 5 | 1 | 1 | | |

The results are presented in consideration of biological significance.

* Significant at significance level of 5% or less

** Significant at significance level of 1% or less (Fisher's exact test)

↑: Significance increases at significance level of 5% or less

↑↑: Significance increases at significance level of 1% or less (Peto/Cochran-Armitage tests)

↓: Significance decreases at significance level of 5% or less

↓↓: Significance decreases at significance level of 1% or less (Cochran-Armitage test)

a) 49 animals were tested. The number of animals tested elsewhere is the same as that specified above.

b) 48 animals were tested. The number of animals tested elsewhere is as specified.

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### II-1-2 Concentrations Administered

The concentrations administered in the carcinogenicity studies were determined based on the results of preliminary studies (2- and 13-week studies).

The maximum concentration for both male and female rats and mice was set at 10,000 ppm, and the lower concentrations were 2,000 ppm and 400 ppm (geometric ratio of 5.0). A group given only deionized water, which was used to dilute the test chemical, was established as the control group.

### II-1-3 Preparation of [the Solutions Containing] the Test Chemical

The test chemical was mixed with drinking water—city water that was deionized, sterilized with ultraviolet light, and filtered—and diluted to the respective concentrations. The concentrations used in the studies were expressed in parts per million (w/w). The solutions were prepared twice weekly at the time the water bottles were changed.

### II-1-4 Concentration Measurement at Time of Preparation

The concentrations of the test chemical in the drinking water were measured with a gas chromatograph.

The concentrations prepared for each group of rats varied in comparison to the set concentration within a range of 81.0 to 120.8% for the 400 ppm group, 89.0% to 102.7% for the 2,000 ppm group, and 71.4% to 112.0% for the 10,000 ppm group, and for the mice, these values ranged from 81.0% to 113.3% for the 400 ppm group, 88.4% to 118.4% for the 2,000 ppm group, and 74.1% to 120.0% for the 10,000 group.

### II-1-5 Stability of the Test Chemical Under Conditions of Administration

The stability of the test chemical in the drinking water was analyzed with a gas chromatograph by measuring the concentration of the preparations around the time of administration to the animals (over a four-day period). A comparison of the results of analysis confirmed stability.

The measured concentrations on day four in comparison to the set concentrations were, for the rats, 80% for the 400 ppm group, 72% for the 2,000 ppm group, and 74% for the 10,000 ppm group and, for the mice, 94% for the 400 ppm group, 96% for the 2,000 ppm group, and 86% for the 10,000 ppm group.

### II-1-6 Amount of the Test Chemical Consumed

The amount of the test chemical consumed per unit body weight (g/kg/day) was calculated from the amount of water consumed during the latter four days of each week on which measurements were taken.

## II-2 Handling of the Animals

### II-2-1 Number of Animals Used in Each Group

Three treated groups and 1 control group were established for a total of four groups, each of which contained 50 males and 50 females for a total of 400 animals for each of the rats and the mice.

### II-2-2 Group Allocation and Identification of Individuals

The animals used in the studies were allocated into the treated groups using an appropriate stratifying method, or in greater detail, they were allocated to minimize body weight differences among the groups by first allocating 1 animal into each group in order of decreasing body weight and then, beginning on the second pass, allocating the animals in order of decreasing body weight into the group with the least total body weight. (See Reference 4.)

The individual animals were identified by marking them with pigment during the quarantine and acclimation periods and by punching their ears during the administration period. In addition, individual identification numbers were affixed to their cages.

The rats and the mice were differentiated from those in other studies and from other animals by keeping them in respective independent rooms surrounded by barriers and by displaying the study number, animal type, and animal numbers on each room.

### II-2-3 Rearing Conditions

Throughout the rearing period, the animals in each study were kept at a temperature of 24±2°C, a humidity of 55±10%, a light/dark cycle of 12 hours of light (8:00 a.m. to 8:00 p.m.) and 12 hours of dark (8:00 p.m. to 8:00 a.m.), and ventilation at 15 to 17 times per hour.

The animals were kept in individual cages (stainless steel double mesh cages, 170 mm W x 294 mm D x 176 mm H for the rats and 112 mm W x 212 mm D x 120 mm H for the mice), and the cages were cleaned once every 2 weeks.

The food given the animals was CRF-1 solid food by Oriental Yeast, Co., Ltd. (sterilized with 3 Mrad of γ radiation), and the animals were allowed to feed freely from a solid food dispenser throughout the rearing period.

The water used was city tap water (supplied by Hadano Municipal Water Works) filtered and then sterilized with ultraviolet radiation. The animals were allowed to drink freely from an automatic water dispenser during the quarantine period and then from a brown glass water dispensing bottle during the acclimation and administration periods. The water bottles were changed twice weekly.

Each time a lot of food was obtained, it was confirmed to be free of abnormalities based on analysis documentation from Oriental Yeast for the nutrients of the food and data from Japan Food Research Laboratories for the impurities. Water quality was confirmed based on analysis data of the Food and Drug Safety Center.

## II-3 Items and Methods of Observations and Testing

### II-3-1 Observation of General Condition of Animals

The general condition of the animals was observed once daily in each study.

### II-3-2 Measurement of Body Weight

The body weight of the animals was measured once weekly from the start of administration to week 14 and then once every other week thereafter. The body weight of dead animals was measured when they were discovered and that of slaughtered animals was measured at the time of slaughtering.

### II-3-3 Measurement of Water Consumption

The amount of water each individual animal consumed was measured twice weekly from the start of administration to week 14 and then once every other week thereafter (during the final four days of a week).

**II-3-4 Measurement of Food Consumption**
The amount of food each individual animal consumed was measured once weekly from the start of administration to week 14 and then once every four weeks thereafter.

**II-3-5 Hematology Testing**
Those animals surviving until the time of the scheduled necropsy were anesthetized with ether immediately before the necropsy, and blood on which hematology testing was to be performed was drawn from the abdominal aorta and collected in a sample tube containing EDTA-2K. The animals subject to this testing were kept from eating for at least 18 hours starting on the day before necropsy. The test variables are listed in Table 1.

**II-3-6 Blood Biochemistry Testing**
Those animals surviving until the time of the scheduled necropsy were anesthetized with ether immediately before the necropsy, and blood was collected in a sample tube containing lithium heparin and centrifuged. The blood plasma thus obtained was used in the blood biochemical testing. The animals subject to this testing were kept from eating for at least 18 hours starting on the day before necropsy. The test variables are listed in Table 1.

**II-3-7 Urinalysis**
Fresh urine was collected from the animals surviving until the last week of administration for use in urinalysis. The test variables are listed in Table 1.

**II-3-8 Pathological Testing**
***(1) Necropsy***
A necropsy was performed on each of the animals.

***(2) Organ Weight***
The actual weights of the organs listed in Table 1 were measured for all animals surviving until the time of the scheduled necropsy. In addition, the ratio of organ weight to body weight (i.e., the percentage of organ weight to body weight at the time of necropsy) was calculated.

***(3) Histopathologic Testing***
The organs from each animal were fixed in a 10% neutral phosphoric acid formalin buffer solution. Then, the organs shown in Table 1 and the tissues with visually apparent changes were embedded in paraffin, cut, stained with hematoxylin-eosin stain, and histopathologically observed under an optical microscope. From the animals on which a necropsy was performed toward the end of the study (week 97 or later for the rats, week 94 or later for the mice), the maxilla in addition to the organs listed in Table 1 was preserved, and a sample was prepared.

## II-4 Data Processing and Statistical Methodology

### II-4-1 Handling and Presentation of Data

Body weight is expressed in grams. That for the rats is given in integers derived by rounding the first number after the decimal point. That for the mice is given to the first decimal place and was derived by rounding the second number after the decimal place.

The amount of food consumed is also given in grams. The amount of food consumed throughout the measurement period was measured to the first decimal place. The value thus derived was divided by the number of days in the measurement period to calculate the daily mean food consumption, with the second number after the decimal place rounded and the values expressed to the first decimal place.

The amount of water consumed is also given in grams. The amount of water consumed throughout the measurement period was measured to the first decimal place. The value thus derived was divided by the number of days in the measurement period to calculate the daily mean water consumption, with the second number after the decimal place rounded and the values expressed to the first decimal place.

The amount of vinyl acetate consumed per unit body weight was determined by multiplying the set concentration of vinyl acetate by the amount of water consumed and dividing by body weight, with the result expressed as g/kg (body weight)/day to the third decimal place, rounding the fourth decimal place.

Actual organ weights were expressed in grams and measured to the third decimal place. Relative organ weight, obtained by dividing the actual organ weight by body weight at the time of necropsy, is expressed in percentage form to the third decimal place, with the fourth decimal place rounded.

The A/G ratio, expressed to the first decimal place with the second decimal place rounded, was obtained using the following formula:

Albumin / (total protein – albumin)

The mean and standard deviation corresponding to each item noted above were rounded so that they contained the same number of decimal places as the corresponding item.

### II-4-2 Handling of Population Size

The body weight and amount of food consumed were measured for each animal living at each respective measurement period, and missing measurements were removed from consideration.

The weight of the organs was measured and hematological and blood biochemistry tests were conducted for all animals surviving to the time of the scheduled necropsies, and missing measurements were removed from consideration.

A urinalysis was conducted on each animal surviving until the final week of administration, and the number of urinalyses was taken as the population size.

The effective number of animals in each group (the number of animals used in the study minus the number of animals removed because of an accident or other reason) was used as the population size for necropsy and histopathological data. The population size used for neoplastic lesion analysis for each organ was taken to be the total number of organs minus the number of organs on which a necropsy could not be performed.

### II-4-3 Statistical Methodology

With the control group used as the standard group, all measurements obtained in the studies were first tested for homogeneity using Bartlett's test. If homogeneity was identified, one-way analysis of variance was performed. If a significant difference was recognized between the groups, the mean values were tested with Dunnett's multiple comparison test. If unequal distribution was identified, the measurements were ranked throughout the groups, a Kruskal-Wallis test was performed, and Dunnett's multiple comparison was performed if a significant difference was noted between any of the groups.

In preliminary testing, two-sided tests were used at a significance level of 5%, and in final testing, two-sided tests at a significance level of 1% and 5% were used.

Data from animals not noted to have a non-neoplastic legion in histopathologic examination were assigned a grade of zero, and an $x^2$ test was performed. An $x^2$ test was also performed for urinalysis.

In the case of neoplastic lesions, Peto tests (Reference 5), the Cochran-Armitage test, and Fisher's exact test were conducted for each tumor based on the total number of afflicted organs in each group. Peto tests were conducted using the scores listed below at the time of histopathological examination for mortality (tests for tumors scored a 3 or 4), prevalence (tests for tumors scored a 0, 1, or 2), and mortality-prevalence (tests with total scores of 0 to 4).

The $x^2$ test and Fisher's exact test compared the control group to each treated group.

Any item for the males or females in any of the groups tested 2 or fewer times was removed from consideration in testing.

Note: Scores used in Peto tests
0: Tumors found in subjects of scheduled necropsies.
1: Tumors found in dead/moribund subjects not directly related to death.
2: Tumors probably but not definitely classifiable as 1.
3: Tumors probably but not definitely classifiable as 4.
4: Tumors found in dead/moribund subjects that were directly related to death.

## III Results

### III-1 Carcinogenicity Study with Rats

#### III-1-1 Observation of Condition of Animals

*(1) Survival*

Animal survival is illustrated in Figs. 1 and 2.

No significant difference between the survival of the treated groups and that of the control group was observed in the males or females.

The number of animals surviving to week 104 (with survival rate in parentheses) was, for the males, 44 of 50 (88%) in the control group, 40 of 50 (80%) in the 400 ppm group, 36 of 50 (72%) in the 2,000 ppm group, and 39 of 50 (78%) in the 10,000 ppm group. For the females, these statistics were: 41 of 50 (82%) in the control group, 40 of 50 (80%) in the 400 ppm group, 41 of 50 (82%) in the 2,000 ppm group, and 37 of 50 (74%) in the 10,000 ppm group.

[Fig. 1]

*(2) General Condition*

The number of animals afflicted with an internal or external tumor as determined from the observation of general conditions is shown in Tables 2 and 3.

Tumors in the oral cavity thought to be due to the administration of vinyl acetate numbered 1 in the 2,000 ppm group and 2 in the 10,000 ppm group for males. For females, there were 1 each in the 400 and 10,000 ppm groups. The incidence of internal and external tumors in the other treated groups did not differ notably from that in the control group for males or females. Observation of dead animals, moribund animals, and animals given the scheduled necropsy revealed no findings characteristic of the administration of the test chemical.

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[Table 3]

***(3) Body Weight***

Figs. 3 and 4 illustrate the transition in body weight.

Both the males and females in the 10,000 ppm group, the group receiving the highest dosage, experienced a slight decrease in weight gain that was at most 8% in the males and 10% in the females in comparison to the control group.

***(4) Water Consumption***

Water consumption is shown in Figs. 5 and 6.

A drop in water consumption was noted in the males and females of the 10,000 ppm group, the group receiving the highest dosage.

Throughout the administration period, males in the 10,000 ppm group consumed on average 83% as much water as their control group counterparts. This figure was 75% for the females.

***(5) Food Consumption***

Food consumption is shown in Figs. 7 and 8.

The amounts of food consumed by the males and females of the treated groups did not differ significantly from that of the control group.

***(6) Amount of Test Chemical Consumed***

The amount of the test chemical consumed (g/kg/day) was calculated from body weight, amount of water consumed, and set concentration.

The daily amount consumed ranged in the males from 0.016 to 0.048 g/kg in the 400 ppm group, 0.075 to 0.226 g/kg in the 2,000 ppm group, and 0.364 to 0.950 g/kg in the 10,000 ppm group. The females consumed from 0.022 to 0.060 g/kg in the 400 ppm group, 0.109 to 0.266 g/kg in the 2,000 ppm group, and 0.478 to 1.062 g/kg in the 10,000 ppm group.

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### III-1-2 Hematological and Blood Biochemistry Tests

*(1) Hematological Tests*

The tests revealed in the males no significant differences between the test and control groups.

In the females, hemoglobin concentration and MCH were elevated in the 400 ppm group, and MCHC was elevated in the 2,000 ppm group, but the increases were only slight and were not dose correlated.

*(2) Blood Biochemistry Tests*

The 10,000 ppm males, who received the maximum concentration, exhibited an elevated A/G ratio and decreased levels of total cholesterol, phospholipids, and calcium.

The females [in the treated groups] did not significantly differ from those of the control group.

*(3) Urinalysis*

Males in the 400 and 10,000 ppm groups experienced decreased urinary pH, and an increase in false positives for ketone bodies was noted in the 10,000 ppm group.

The females [in the treated groups] did not significantly differ from those of the control group.

### III-1-3 Pathological Testing

*(1) Necropsies*

Mandibular nodes were noted in 3 of 50 males in the 10,000 ppm group and in 1 of 50 females in each of the 400 and 10,000 ppm groups. The incidence of granulation of the kidneys was inversely proportional to the concentration administered (16 of 50 in the control group, 18 of 50 in the 400 ppm group, 11 of 50 in the 2,000 ppm group, and 6 of 50 in the 10,000 ppm group).

*(2) Organ Weights*

A decrease in the actual weights of the kidneys and livers of the males in the 10,000 ppm group was noted, but this is thought to be a change accompanying the low body weight of these animals at the time of necropsy.

*(3) Histopathologic Testing*

Tables 4 and 5 list major neoplastic lesions and associated non-neoplastic lesions.

*—Major Neoplastic Lesions—*

Oral Cavity

Peto tests (mortality, prevalence, and mortality-prevalence) and the Cochran-Armitage test indicated an increased incidence of squamous cell carcinoma in the males (control group: 0/50; 400 ppm group: 0/50; 2,000 ppm group: 0/50; 10,000 ppm group: 5/50), and Fisher's exact test also confirmed an increase in the 10,000 ppm group over the control group. Moreover, squamous cell papilloma was noted in 2 of the 50 members of the 10,000 ppm group. The incidence of squamous cell papilloma and carcinoma, when considered together, (control group: 0/50; 400 ppm group: 0/50; 2,000 ppm group: 0/50; 10,000 ppm group: 7/50) also showed an increase using Peto tests (mortality, prevalence, and mortality-prevalence) and the Cochran-Armitage test, and again, Fisher's exact test also confirmed an increase in the 10,000 ppm group over the control group. In the females, a Peto test (prevalence) indicated an increase in the incidence of squamous cell carcinoma (control group: 0/50; 400 ppm group: 1/50; 2,000 ppm group: 1/50; 10,000 ppm group: 3/50). (See Tables 4, 5, 6, and 7.)

Squamous cell carcinoma metastasis to the tongue was noted in 1 of the 50 males in the 10,000 ppm group.

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[Table 7]

Esophagus
Squamous cell carcinoma was noted in 1 of the 50 females of the 10,000 ppm group (Tables 4 and 5).

—Other Neoplastic Lesions—
A Peto test (prevalence) revealed an increasing trend in the occurrence of interstitial cell tumors in the testes of males (control group: 42/50; 400 ppm group: 40/50; 2,000 ppm group: 44/50; 10,000 ppm group: 47/50), but as the incidence rate in the 10,000 ppm group fell within the range of the historical control data of this Center (mean 89.6% with individual studies finding rates of 82 to 98%; Reference 6), the administration of the test chemical is not considered to have been influential (Table 8).

Furthermore, the occurrence of adenocarcinoma in the mammary glands of females was determined to have increased using Peto tests (prevalence and mortality-prevalence) and the Cochran-Armitage test (control group: 0/50, 400 ppm group: 0/50; 2,000 ppm group: 0/50; 10,000 ppm group: 3/50). The incidence rate in the 10,000 ppm group, however, fell within the range of the historical control data of this Center (mean 2.0% with individual studies finding rates of 0 to 6%; Reference 6), so the administration of the test chemical is not considered to have been influential (Table 9).

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Fisher's exact test revealed a significant difference between the control and 2,000 ppm groups in the incidence of C-cell adenomas and C-cell carcinomas in the thyroid glands of females (control: 2/50; 400 ppm group: 7/50; 2,000 ppm group: 9/50; 10,000 ppm group: 6/50). (See Table 10.) But as this change did not correlate to concentration, it is not thought to be due to the administration of the test chemical.

[Table 10]

—Non-neoplastic Lesions—
Oral Cavity
In the 10,000 ppm group, basal cell activation was noted in 2 of the 50 males and 1 of the 50 females, and in the same group, epithelial dysplasia was observed in 2 of the 50 females (Tables 4 and 5).

Esophagus
In the 10,000 ppm group, basal cell activation was seen in four of the 50 females, and squamous cell hyperplasia was noted in 1 male and 1 female (Tables 4 and 5).

Stomach
Activation of the basal cells of the proventriculus was noted in 2 of the 50 males and five of the 50 females in the 10,000 ppm group. The 10% incidence rate in the 10,000 ppm females constituted a statistically significant increase over the 0% rate in the control group (Tables 4 and 5).

Kidneys
The scheduled necropsies revealed a decrease in the extents of chronic nephropathy in the 10,000 ppm males.

—Other Non-neoplastic Lesions—
An increase in the extent of eosinophilic change in the olfactory epithelium in the nasal cavity was noted in dead and moribund females in the 10,000 ppm group, and a decrease in the incidence of cell growth in the adrenal medulla was seen in dead and moribund males in the 2,000 and 10,000 ppm groups. These findings, however, were not made during the scheduled necropsies, so their relationship to the test chemical is unknown. Furthermore, the extent of bile duct growth in the liver tapered off in the 10,000 ppm females as observed during the scheduled necropsies, but the relationship of this finding to the test chemical is unknown. Other non-neoplastic lesions occurring at a rate not statistically significantly different from that of the control group were noted. There was a drop in the respiratory epithelialization of the nasal glands and an increase in retinal atrophy in males undergoing the scheduled necropsy and a decrease in clear cell growth sites in the liver of females. The incidence rates of these findings, however, were not concentration dependent, and are as such not thought to be due to the administration of the test chemical.

***(4) Causes of Death***
The causes of death and moribundity from a pathological standpoint are presented in Table 11. No significant difference was noted among the groups in this area.

[Table 11]

## III-2 Carcinogenicity Study With Mice

### III-2-1 Observation of Condition of Animals

*(1) Survival*

Survival is illustrated in Figs. 9 and 10.

No significant difference between the survival rates of each of the treated groups and the control group was noted for either the males or females.

The numbers of animals surviving (and survival rates) over the 104-week period in each group were as follows: Males: control: 35/50 (70%); 400 ppm group: 42/50 (84%); 2,000 ppm group: 38/50 (76%); 10,000 ppm group: 33/50 (66%); and females: control: 26/50 (52%), 400 ppm group: 27/50 (54%); 2,000 ppm group: 25/50 (50%); 10,000 ppm group: 23/50 (46%).

[Fig. 9]

[Fig. 10]

***(2) General Condition***

The number of animals noted to have an internal or external tumor during general observations is shown in Tables 12 and 13.

Oral tumors thought to have been caused by the administration of vinyl acetate were observed in 6 males and 6 females in the 10,000 ppm group, which received the highest concentration. Apart from this, there was no significant difference in external tumor incidence between each treated group and the control group. An observation of the incidence of internal tumors in all the male animals (dead or moribund animals; those subjected to the scheduled necropsy) reveals 10 occurrences of the 50 in the control group, 18 of 50 in the 400 ppm group, and 5 of 50 in the 10,000 ppm group, which gives significantly different numbers of incidence that are, however, not proportional to concentration. In the females, no significant difference in the incidence of internal tumors was noted. Other general observations did not reveal any findings specific to vinyl acetate in the dead or moribund mice and the ones subjected to the scheduled necropsies.

***(3) Body Weight***

Figs. 11 and 12 illustrate the transition in body weight.

A repressed weight gain was noted in both the males and females of the 10,000 ppm group, which received the highest concentration. The repression of body weight in these groups below the control was at most 30% for the males and 18% for the females.

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*(4) Water Consumption*

Water consumption is shown in Figs. 13 and 14.

Decreased water consumption was noted in the males in only the 10,000 ppm group and in a dose-dependent manner in the females in all treated groups.

The mean water consumption of each of these groups as a percentage of the consumption by the control group was as follows: males: 90% in the 10,000 ppm group; females: 96% in the 400 ppm group, 92% in the 2,000 ppm group, and 84% in the 10,000 ppm group.

*(5) Food Consumption*

The amount of food the animals consumed is shown in Figs. 15 and 16.

There were no significant differences in the amounts consumed by any of the treatment groups and the control groups for either the males or females.

*(6) Amount of Test Chemical Consumed*

The amount of the test chemical consumed was determined from body weight, amount of water consumed, and the set concentration of the test chemical.

The ranges of daily consumption of the test compound were, for the males, 0.032 to 0.085 g/kg in the 400 ppm group, 0.167 to 0.405 g/kg in the 2,000 ppm group, and 0.800 to 2.081 g/kg in the 10,000 ppm group. For the females, these ranges were 0.045 to 0.125 g/kg in the 400 ppm group, 0.230 to 0.483 g/kg in the 2,000 ppm group, and 1.024 to 2.185 g/kg in the 10,000 ppm group.

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### III-2-2 Hematological and Blood Biochemistry Testing and Urinalysis

*(1) Hematological Testing*

The males in the 10,000 ppm group, which received the highest concentration, experienced elevated platelet counts and lobed-nucleus neutrophils as well as a decreased lymphocyte levels.

The females of this group experienced decreased MCHC.

*(2) Blood Biochemistry Testing*

In the males of the 400 and 10,000 ppm groups, decreased glucose was noted, and in the 10,000 ppm group, increased A/G ratios, increased ALP activity, and decreased total cholesterol, triglyceride, and calcium levels were observed.

In the females of the 10,000 ppm group, a decrease in glucose was noted.

*(3) Urinalysis*

The males of the 10,000 ppm group experienced a decrease urinary pH and increased urinary protein.

In the females of this group, increased urinary protein and ketone bodies were noted.

### III-2-3 Pathological Testing

*(1) Necropsies*

The necropsies of the 10,000 ppm group revealed mandibular nodes in 1 of the 50 males and 5 of the 50 females and maxillary nodes in 3 of the 50 males and 1 of the 50 females.

*(2) Organ Weights*

Decreased body weight, as determined at the time of necropsy, in the males of the 10,000 ppm group brought with it a decrease in the actual weights of the heart, lungs, kidneys, and liver and an increase in the weights of the testes, heart, lungs, kidneys, liver, and brain relative to body weight.

In the females of the 10,000 ppm group, an increase in the weights of the lungs, kidneys, and brain relative to body weight was noted, but this change was thought to be due to decreased body weight as measured at the time of necropsy.

*(3) Histopathologic Testing*

The major neoplastic lesions and the associated non-neoplastic lesions are listed in Tables 14 and 15.

—Major Neoplastic Lesions—

Oral Cavity

Peto tests (mortality, prevalence, and mortality-prevalence) and the Cochran-Armitage test revealed an increasing trend in the incidence of squamous cell carcinoma in the males (control: 0/50; 400 ppm group: 0/50; 2,000 ppm group: 0/50; 10,000 ppm group: 13/50), and Fisher's exact test indicated a significant increase in incidence in the 10,000 ppm group over the control group. The incidence of squamous cell papilloma (control: 0/50; 400 ppm group: 0/50; 2,000 ppm group: 0/50; 10,000 ppm group: 4/50) also showed an increasing trend by a Peto test (prevalence) and the Cochran-Armitage test. The combined incidences of squamous cell carcinoma and squamous cell papilloma (control: 0/50; 400 ppm group: 0/50; 2,000 ppm group: 0/50; 10,000 ppm group: 16/50) as well were found to have in increasing trend by Peto tests (mortality, prevalence, and mortality-prevalence) and the Cochran-Armitage test, and Fisher's exact test again indicated a significant increase in the 10,000 ppm group.

[Table 14]

In the females, Peto tests (mortality, prevalence, and mortality-prevalence) and the Cochran-Armitage test revealed an increasing trend in the incidence of squamous cell carcinoma (control: 0/50; 400 ppm group: 0/50; 2,000 ppm group: 0/50; 10,000 ppm group: 15/49), and Fisher's exact test indicated a significant increase in incidence in the 10,000 ppm group over the control group. The incidence of squamous cell papilloma (control: 0/50; 400 ppm group: 0/50; 2,000 ppm group: 0/50; 10,000 ppm group: 3/49) also showed an increasing trend by a Peto test (prevalence) and the Cochran-Armitage test. The combined incidences of squamous cell carcinoma and squamous cell papilloma (control: 0/50; 400 ppm group: 0/50; 2,000 ppm group: 0/50; 10,000 ppm group: 18/49) as well were found to have in increasing trend by Peto tests (mortality, prevalence, and mortality-prevalence) and the Cochran-Armitage test, and Fisher's exact test again indicated a significant increase in the 10,000 ppm group.

Two males and two females in the 10,000 ppm group experienced metastasis of squamous cell carcinoma to the lungs and lymph nodes, and one female in this group experienced metastasis to the salivary glands (Tables 14, 15, 16, and 17).

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Esophagus
The incidence of squamous cell carcinoma in the males (control: 0/50; 400 ppm group: 0/50; 2,000 ppm group: 0/50; and 10,000 ppm group: 7/50) was found to have an increasing trend by Peto tests (prevalence and mortality-prevalence) and the Cochran-Armitage test, and Fisher's exact test revealed a significant increase in the 10,000 ppm group over the control group. In the females, squamous cell papilloma was noted in 1 of 50 members of the 2,000 ppm group, and squamous cell carcinoma was seen in 1 of the 50 members of the 10,000 ppm group. Squamous cell carcinoma metastasized to the lungs of 1 male in the 10,000 ppm group (Tables 14, 15, and 18).

[Table 18]

Stomach
In the males, the incidence of squamous cell carcinoma (control: 1/50; 400 ppm group: 0/50; 2,000 ppm group: 0/50; and 10,000 ppm group: 7/50) was shown to have an increasing trend using Peto tests (prevalence and mortality-prevalence) and the Cochran-Armitage test, and Fisher's exact test revealed a significant increase in the 10,000 ppm group over the control group. Squamous cell papilloma was noted in 2 of the 50 members of the 10,000 ppm group. The incidence of squamous cell carcinoma and papilloma together (control: 1/50; 400 ppm group: 0/50; 2,000 ppm group: 0/50; and 10,000 ppm group: 9/50) was also shown to exhibit an increasing trend using Peto tests (prevalence and mortality-prevalence) and the Cochran-Armitage test, and Fisher's exact test again revealed a significant increase in the 10,000 ppm group over the control group.

In the females, the incidence of squamous cell carcinoma (control: 0/50; 400 ppm group: 0/50; 2,000 ppm group: 0/50; and 10,000 ppm group: 3/49) was shown to have an increasing trend using Peto tests (mortality, prevalence, and mortality-prevalence) and the Cochran-Armitage test. Squamous cell papilloma was noted in 1 of the 50 members of the 10,000 ppm group. The incidence of squamous cell carcinoma and papilloma together (control: 0/50; 400 ppm group: 0/50; 2,000 ppm group: 0/50; and 10,000 ppm group: 4/49) was also shown to exhibit an increasing trend using Peto tests (mortality, prevalence, and mortality-prevalence) and the Cochran-Armitage test. All tumors originated in the proventriculus. Squamous cell carcinoma metastasized to the lungs of 1 male and 1 female of the 10,000 ppm group and to the kidneys, pancreas, and lymph nodes of a female in the same group (Tables 14, 15, 19, and 20).

[Table 19]

[Table 20]

Larynx
Squamous cell carcinoma occurred in 1 of the 50 females in the 2,000 ppm group and 2 of the 50 males and 1 of the 50 females of the 10,000 ppm group. The female in the 2,000 ppm group was the same individual noted above to have a squamous cell papilloma in her esophagus (Tables 14 and 15.)

Liver
The incidence of hepatocyte carcinoma in the males (control: 13/50; 400 ppm group: 10/50; 2,000 ppm group: 9/50; 10,000 ppm group: 4/50) exhibited a decreasing trend according to the Cochran-Armitage test, and incidence was significantly lower in the 10,000 ppm group than in the control group according to Fisher's exact test (Table 21).

[Table 21]

—Non-neoplastic Lesions—
Oral Cavity
Squamous cell hyperplasia was noted in 2 of the 50 males and 1 of the 50 females in the 2,000 ppm group and 13 of the 50 males and 6 of the 50 females of the 10,000 ppm group. Basal cell activation was seen in 1 male and 1 female in the 2,000 ppm group and 18 of 50 males and 17 of 49 females in the 10,000 ppm group. In addition, epithelial dysplasia was recognized in 24 of the 50 males and 17 of the 49 females in the 10,000 ppm group. These findings in the males and females were all discovered during the scheduled necropsies, and there was a statistically significant difference between the control and 10,000 ppm groups (Tables 14 and 15).

Esophagus
Basal cell activation was seen in 9 of the 50 males and 15 of the 49 females in the 10,000 ppm group. Squamous cell hyperplasia was noted in 2 of the 49 females in the 10,000 ppm group. Finally, epithelial dysplasia was recognized in 2 of the 50 males and 7 of the 49 females [in the 10,000 ppm group]. Basal cell activation was noted in both the males and females in the scheduled necropsies and in dead or moribund animals, and there was a statistically significant difference between the control and 10,000 ppm groups (Tables 14 and 15).

Stomach
Basal cell activation was noted in 1 of the 50 males and 1 of the 49 females in the 10,000 ppm group, and epithelial dysplasia was seen in 1 of the 50 males in the 10,000 ppm group (Tables 14 and 15).

In the males subjected to scheduled necropsies, the incidence of glandular stomach hyperplasia (control: 25/35; 400 ppm group: 31/42; 2,000 ppm group: 26/38; 10,000 ppm group: 11/33) was significantly lower in the 10,000 ppm group than in the control group.

Hyperplasia of the proventriculus was noted in males and females of the 10,000 ppm group and females of the 400 ppm group. This finding, however, could be an age-related change and therefore could not be attributed to the administration of the vinyl acetate.

Larynx
Basal cell activation was noted in 3 of the 50 males and 6 of the 49 females in the 10,000 ppm group, epithelial dysplasia was seen in 2 of the 50 males and 3 of the 49 females in the 10,000 ppm group, and squamous cell hyperplasia was confirmed in 1 of the 50 males [in the 10,000 ppm group]. The incidence of basal cell activation as determined in the scheduled necropsies of the females was statistically significantly higher in the 10,000 ppm group (Tables 14 and 15).

Salivary Glands
Atrophy of the salivary glands was noted in 6 of the 50 males and 4 of the 49 females in the 10,000 ppm group.

Nasal Cavity
The scheduled necropsies revealed for the incidence of respiratory epithelialization of the nasal glands in males (control: 26/35; 400 ppm group: 28/42; 2,000 ppm group: 26/38; 10,000 ppm group: 12/33) a statistically significant decrease in the 10,000 ppm group.

Brain
The incidence of mineral deposits in males (control: 8/50; 400 ppm group: 17/50; 2,000 ppm group 16/50; 10,000 ppm group: 19/50) was determined from the scheduled necropsies to be statistically significantly higher in the 10,000 ppm group.

—Other Non-neoplastic Lesions—
Other non-neoplastic lesions identified in males subjected to scheduled necropsy were a decrease in basophilic change in the kidneys, an increase in vacuolization of the proximal renal tubular epithelium, and an increase in mineral deposits in the testes. In the females, there were increases in eosinophilic change in the respiratory epithelium of the nasal cavity and increased cystic endothelial growth in the uterus. The findings, however, did not exhibit concentration dependency and are therefore not thought to be due to the administration of the test chemical.

*(4) Causes of Death*

The causes of death and moribundity from a pathological standpoint are presented in Table 22.

Six males and 4 females in the 10,000 ppm group died of oral tumors. A few males and females in the 10,000 ppm group died of tumors in the stomach or larynx. Finally, 1 male in the 10,000 ppm group died of a tumor in the esophagus.

[Table 22]

## IV. Discussion

### IV-1 Rats

—Survival and Other Aspects—
There was no significant difference between the respective survival rates of the treated groups and the control group for either males or females. Weight gain was slightly reduced in the males and females of the 10,000 ppm group, and water consumption was lower. Also seen in both sexes in the 10,000 ppm group were, in blood biochemistry testing, decreased total cholesterol and phospholipids and, in urinalysis, an increase in false positives for ketl bodies and other mild nutritional disorders.

—Neoplastic Lesions—
***(1) Oral Cavity***
In the males of the 10,000 ppm group, there were 5 occurrences of squamous cell carcinoma and 2 cases of squamous cell papilloma. The historical control data of this Center on F344 rats (Reference 6) contains no mention of oral squamous cell carcinoma and only 1 rare instance in 550 (0.2%) of squamous cell papilloma. These cancers are therefore thought to be a result of the administration of vinyl acetate. Of the individuals afflicted with squamous cell carcinoma, 1 suffered an invasive metastasis into the esophagus. This particular cancer was progressive and had a high malignancy.

The females suffered squamous cell carcinoma at an incidence of 1 in 50 in the 400 ppm group, 1 in 50 in the 2,000 ppm group, and 3 in 50 in the 10,000 ppm group. The historical control data of this Center on the incidence of oral squamous cell carcinoma (Reference 6) and NTP data (Reference 7) contains no mention of this carcinoma, which is very rare and likely due to the administration of vinyl acetate.

***(2) Esophagus***
Squamous cell carcinoma was noted in 1 of the 50 females in the 10,000 ppm group. This type of cancer, which was not noted in the historical control data of this Center (Reference 6) or the NTP data (Reference 7), is very rare. It is therefore thought to have occurred as a result of the administration of the vinyl acetate.

—Non-neoplastic Lesions—
The scheduled necropsies of the 10,000 ppm group revealed activation of the basal cells in the proventriculus in 5 of the 50 females, which marks a statistically significant increase over the control group and is thought to be due to the administration of vinyl acetate. Also in this group were noted activation of the basal cells (2 males and 1 female) and epithelial dysplasia (2 females) of the oral cavity, activation of the basal cells (4 females) and squamous cell hyperplasia (1 male and 1 female) in the esophagus, and activation of the basal cells of the proventriculus (2 males). Although these changes did not occur statistically significantly more often than in the control group, they are not changes associated with aging and, as with tumors in the oral cavity and esophagus, were limited to the squamous epithelium of the digestive organs, and as such, they are considered to be due to the administration of vinyl acetate.

A decrease in the extent of chronic nephropathy in the kidneys of the 10,000 ppm males was noted. Chronic nephropathy, a common result of aging in F344 rats, is reported to be repressed by limiting food intake (Reference 8). In this study, however, food consumption was not severely limited, and no great repression of weight gain was noted, so the cause of this phenomenon is not considered to be restricted food intake.

Hyperplasia in the proventriculus, squamous hyperplasia of a digestive organ such as the mouth and esophagus, was noted in one 2,000 ppm male. But hyperplasia in the proventriculus is a commonly noted age-associated change, and two occurrences were noted in the control group, so this is not considered to be due to administration of the vinyl acetate.

**IV-2 Mice**

The survival rates of the treated groups did not differ significantly from that of the control group for either males or females.

Many deaths caused by oral tumors were noted in the males and females in the 10,000 ppm group. A few males and females of that group died of tumors in the stomach or larynx, and males in the group died of tumors in the esophagus.

Males and females of the 10,000 ppm group suffered reduced body weight gain, and males in the 10,000 ppm group and females in all treatment groups consumed less water. Blood biochemistry testing revealed low glucose, total cholesterol, and triglycerides in males of the 10,000 ppm group and decreased glucose in the females of this group. Through urinalysis, it was discovered that males of the 10,000 ppm group experienced decreased urinary pH levels and increased positive results for urinary protein. The females of this group experienced increased positive results for urinary protein and ketone bodies. A nutritional impairment is suspected as the cause.

—Neoplastic Lesions—

***(1) Oral Cavity***

Squamous cell carcinoma occurred in 13 of the 50 males in the 10,000 ppm group, and squamous cell papilloma occurred in 4 of 50 of these individuals. Moreover, 15 of the 49 females of this group contracted squamous cell carcinoma, and 3 of the 49 developed squamous cell papilloma. These tumors are not listed in the historical control data of this Center (Reference 6) and are very rare. They are therefore thought to have been caused in both the males and females by the administration of vinyl acetate. Some of the squamous cell carcinomas were noted to have metastasized to the lungs, salivary glands, and nearby lymph nodes. One mouse experienced metastasis to a plurality of organs.

*(2) Esophagus*
Squamous cell carcinoma occurred in 7 of the 50 males in the 10,000 ppm group, and these incidences were thought to be due to the administration of vinyl acetate. One of these incidences metastasized.

One of the 50 females in the 2,000 ppm group developed squamous cell papilloma, and 1 of the 50 females in the 10,000 ppm group contracted squamous cell carcinoma. These tumors are not listed in the historical control data of this Center (Reference 6) and rarely occur naturally (References 6, 9, and 10). They are therefore thought to have been caused by the administration of the vinyl acetate.

*(3) Stomach*
One of the 50 males in the control group and 7 of the 50 males in the 10,000 ppm group developed squamous cell carcinoma in the proventriculus, and squamous cell papilloma was noted in 2 of the 50 males in the 10,000 ppm group. Among the females, squamous cell carcinoma occurred in 3 of the 49 members of the 10,000 ppm group, and 1 of 50 developed squamous cell papilloma. As these tumors rarely occur naturally (References 6, 9, and 10), they are thought to be due to the administration of the vinyl acetate. Many of the squamous cell carcinomas grew invasively, and one case metastasized to another organ in a male and in a female. The female noted here experienced widespread intraperitoneal metastasis. Her cancer, considered highly malignant, moved to her liver, kidneys, pancreas, and nearby lymph nodes.

*(4) Larynx*
Squamous cell carcinoma was noted in 2 of the 50 males and 1 of the 49 females in the 10,000 ppm group, and squamous cell papilloma was discovered in 1 of the 50 females of the 2,000 ppm group. Although there were few incidences, these tumors rarely occur naturally (References 6, 9, and 10) and are therefore thought to have been caused by the administration of the vinyl acetate.

*(5) Liver*
The incidence of hepatocyte carcinoma in the males (control: 13/50; 400 ppm group: 10/50; 2,000 ppm group: 9/50, 10,000 ppm group: 4/50) exhibited a decreasing trend according to the Cochran-Armitage test, and Fisher's exact test indicated a significant decrease in the 10,000 ppm group below the control group.

It is reported that liver tumor incidence in female mice is suppressed by weight gain repression (Reference 11). In this study, the administration of the vinyl acetate caused the animals to avoid drinking water, which brought about a reduction in weight gain. This is thought to have brought about a reduction in hepatocyte carcinoma.

There was no significant difference between the incidence of this type of cancer between the females of each treated group and those of the control group.

—Non-Neoplastic Lesions—
The administration of the vinyl acetate is thought to have brought about the following in the 10,000 ppm group: squamous cell hyperplasia, basal cell activation, and epithelial dysplasia in the oral cavity, esophagus, and larynx; and basal cell activation and epithelial dysplasia in the proventriculus.

The number of incidences varied by organ type and was most common in the oral cavity. A few incidences of squamous cell hyperplasia and basal cell activation in the oral cavity were also noted in the 2,000 ppm group. Another example is atrophy of the salivary glands in the males and females of the 10,000 ppm group, but this atrophy occurred in individuals afflicted with tumors on the mandible and is therefore thought to be a secondary change accompanying tumor development on the mandible.

Other findings occurring in a concentration-dependent manner for which the influence of the administration of vinyl acetate could not be ruled out as a contributing cause are as follows:

- Increased incidence of mineral deposits in the brain (in scheduled necropsy on males in 10,000 ppm group)
- Decreased incidence of respiratory epithelialization of the nasal glands (in scheduled necropsy on males in 10,000 ppm group)
- Decreased incidence of glandular stomach hyperplasia (in scheduled necropsy on males in 10,000 ppm group)

**IV-3 Comparison with Other Studies and Wrap-up**

Bogdanffy et al. reported the occurrence of squamous cell carcinoma in the nasal cavity in a vinyl acetate inhalation carcinogenicity study (Reference 12). In the present studies as well, in which vinyl acetate was given in drinking water, squamous-cell-based tumors occurred in the areas put into direct contact with the vinyl acetate—the oral cavity, esophagus, stomach, and the larynx, which adjoins the oral cavity. In both cases, vinyl acetate caused tumors locally in the areas it contacted directly. Lijinsky et al. reported the incidence of C-cell adenoma in the liver, uterus, and thyroid gland in a 100-week vinyl acetate drinking water study (Reference 15), but in the present studies, no increase in tumors attributable to the administration of vinyl acetate was noted in the liver, uterus, or thyroid gland. The similarities and differences in the results may be due to differences in testing format, concentrations administered, and animal number, but Lijinsky et al. fail to elaborate on the testing conditions, so a thorough investigation is not possible.

Non-neoplastic lesions—squamous cell hyperplasia, basal cell activation, and epithelial dysplasia—were observed in the organs that made direct contact with the vinyl acetate when the water was consumed.

With regard to the above findings:

1. These locations correspond to the organs in which increased tumor incidence was seen.
2. The location of incidence was squamous epithelium, the same tissue type from which tumors of increased incidence originated.
3. It is reported that the chemically induced occurrence of squamous cell hyperplasia and basal cell activation—which represent growth-related cellular change—bring about continuous cell growth stimulation and may cause squamous-cell-based tumors (Reference 13) and epithelial dysplasia represents a precancerous lesion that contributes greatly to cell malignancy (Reference 14). As such, it is believed that squamous cell hyperplasia, basal cell activation, and epithelial dysplasia are changes that represent the early stages of squamous cell carcinoma and squamous cell papilloma caused by the administration of vinyl acetate.

## V. Conclusion

Two-year (104-week) carcinogenicity studies in which vinyl acetate was orally given in drinking water to F344/DuCrj (Fisher) rats and CRJ:$BDF_1$ mice were conducted.

In the rats, an increased incidence of squamous cell carcinoma and squamous cell papilloma of the oral cavity was noted in males, and in females, there was an increased incidence of squamous cell carcinoma in the oral cavity and esophagus. The concentrations that brought about oral tumors were 10,000 ppm for the males and 400 ppm and above for the females. This concentration for tumors in the esophagus in females was 10,000 ppm.

In the mice, an increased occurrence of squamous cell carcinoma and squamous cell papilloma of the oral cavity and stomach and squamous cell carcinoma in the esophagus and larynx was noted among the males. In the females, an increased incidence of squamous cell carcinoma and squamous cell papilloma in the oral cavity and stomach and squamous cell carcinoma in the esophagus and larynx was observed. The concentration that brought about oral and stomach tumors was 10,000 ppm for both the males and females. Tumors in the esophagus and larynx occurred in males at 10,000 ppm and in females at 2,000 ppm.

The above results clearly indicate the carcinogenicity that vinyl acetate poses for F344/DuCrj (Fisher) rats and CRJ:$BDF_1$ mice.

**IV. Bibliography**
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Photograph 1
Mandibular nodes (oral squamous cell carcinoma) (↑)
Male rat in 10,000 ppm group. Animal no. 0162-1302.

Photograph 2—Oral Cavity
Squamous cell carcinoma (↑)
Female rat in 10,000 ppm group. Animal no. 0162-2304.
(Hematoxylin-eosin staining; x 32)

Photograph 3—Oral Cavity
Squamous cell papilloma (↑)
Male rat in 10,000 ppm group. Animal no. 0162-2320.
(Hematoxylin-eosin staining; x 80)

Photograph 4—Esophagus
Squamous cell carcinoma (↑)
Female rat in 10,000 ppm group. Animal no. 0162-2304.
(Hematoxylin-eosin staining; x 80)

Photograph 5—Oral Cavity
Epithelial dysplasia (↑)
Female rat in 10,000 ppm group. Animal no. 0162-2304.
(Hematoxylin-eosin staining; x 32)

Photograph 6—Stomach (proventriculus)
Basal cell activation (↑)
Male rat in 10,000 ppm group. Animal no. 0162-1314.
(Hematoxylin-eosin staining; x 80)

Photograph 7
Mandibular node (oral squamous cell carcinoma) (↑)
Female mouse in 10,000 ppm group. Animal no. 0163-2305.

Photograph 8
Mandibular node (oral squamous cell carcinoma) (A)
Lung node (metastasized oral tumor) (B)
Male mouse in 10,000 ppm group. Animal no. 0163-1336.

Photograph 9—Oral Cavity
Oral squamous cell carcinoma (↑)
Male mouse in 10,000 ppm group. Animal no. 0163-1342.
(Hematoxylin-eosin staining; x 16)

Photograph 10—Lungs
Metastasis: Oral tumor (↑)
Female mouse in 10,000 ppm group. Animal no. 0163-1336.
(Hematoxylin-eosin staining; x 80)

Photograph 11—Esophagus
Squamous cell carcinoma (outward growth) (↑)
Male mouse in 10,000 ppm group. Animal no. 0163-1317.
(Hematoxylin-eosin staining; x 16)

Photograph 12—Esophagus
Squamous cell carcinoma (inward growth) (↑)
Male mouse in 10,000 ppm group. Animal no. 0163-1349.
(Hematoxylin-eosin staining; x 32)

Photograph 13—Stomach (proventriculus)
Squamous cell carcinoma (↑)
Female mouse in 10,000 ppm group. Animal no. 0163-2334.
(Hematoxylin-eosin staining; x 16)

Photograph 14—Liver
Metastasized gastric tumor (↑)
Female mouse in 10,000 ppm group. Animal no. 0163-2332.
(Hematoxylin-eosin staining; x 80)

Photograph 15—Larynx
Squamous cell carcinoma (↑)
Male mouse in 10,000 ppm group. Animal no. 0163-1326.
(Hematoxylin-eosin staining; x 16)

Photograph 16—Oral Cavity
Squamous cell hyperplasia (A) and basal cell activation (B)
Male mouse in 10,000 ppm group. Animal no. 0163-1342.
(Hematoxylin-eosin staining; x 32)

Photograph 17—Esophagus
Epithelial dysplasia (↑)
Female mouse in 10,000 ppm group. Animal no. 0163-2331.
(Hematoxylin-eosin staining; x 80)

Photograph 18—Larynx
Epithelial dysplasia (A) and basal cell activation (B)
Female mouse in 10,000 ppm group. Animal no. 0163-2314.
(Hematoxylin-eosin staining; x 80)

MR 58819

# 酢酸ビニルのラット及びマウスを用いた経口投与によるがん原性試験(混水試験)報告書



平成７年１１月３０日

(縮　刷　版)

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要約

酢酸ビニルのがん原性を検索する目的でラットとマウスを用いて経口投与（混水）による2年間（104週間）の試験を実施した。

試験に使用した動物はF344/DuCrj(Fischer)ラットとCrj:BDF$_1$マウスで、雌雄各群とも50匹とし、被験物質投与群3群と対照群1群の計4群の構成で、ラット、マウスともに計400匹を用いた。

投与は、酢酸ビニルを各投与濃度に希釈調製した飲水の自由摂取で行った。投与濃度は、ラット、マウスの雌雄とも 400ppm、2000ppm、10000ppm（公比5.0）とした。観察、検査項目は、一般状態の観察、体重、摂水量、摂餌量の測定、血液学的検査、血液生化学的検査、尿検査、剖検、臓器重量測定及び病理組織学的検査を行った。

ラットでは、雄の 10000ppm群の口腔に扁平上皮癌と扁平上皮乳頭腫、雌の400ppm以上の群の口腔と 10000ppm群の食道に扁平上皮癌が発生した。

また、腫瘍の前段階と考えられる病変として、雌雄とも 10000ppm群の口腔、食道及び胃の扁平上皮に扁平上皮過形成、基底細胞の賦活化及び上皮の異形成が観察された。

マウスでは、雌雄とも 10000ppm群の口腔と胃に扁平上皮癌や扁平上皮乳頭腫、食道と喉頭に扁平上皮癌が発生した。2000ppm群でも少数例ではあるが、雌に食道の扁平上皮乳頭腫と喉頭の扁平上皮癌の発生が認められた。また、口腔に近接した喉頭に扁平上皮癌の発生が、雄では 10000ppm群に、雌では2000ppm、10000ppm群に認められた。腫瘍の前段階と考えられる病変として、雌雄とも 10000ppm群で扁平上皮過形成、基底細胞の賦活化及び上皮の異形成が、口腔、食道、胃及び喉頭の被蓋上皮である扁平上皮に観察された。また、2000ppm群でも少数例ではあるが口腔の扁平上皮に過形成、基底細胞の賦活化及び上皮の異形成が認められた。

以上により、酢酸ビニルの2年間にわたる飲水による経口投与試験の結果、F344/DuCrj (Fisher) ラットの雄に口腔の扁平上皮癌と扁平上皮乳頭腫、雌に口腔と食道の扁平上皮癌を発生増加させ、酢酸ビニルの癌原性が証明された。

また、Crj:BDF$_1$マウスでは、雌雄に口腔と胃の扁平上皮癌と、扁平上皮乳頭腫、食道と喉頭の扁平上皮癌の発生増加が認められ、また、雌に食道の扁平上皮乳頭腫が認められた。これにより酢酸ビニルのがん原性が証明された。

酢酸ビニルのがん原性試験における主な腫瘍発生（ラット：雄）

| | 投与濃度（ppm） | | 0 | 400 | 2000 | 10000 | ペトー検定 | コクランアミテージ検定 |
|---|---|---|---|---|---|---|---|---|
| | 検査動物数 | | 50 | 50 | 50 | 50 | | |
| 良性腫瘍 | 皮膚 | 角化棘細胞腫 | 3 | 2 | 4 | 1 | | |
| | 皮下組織 | 線維腫 | 3 | 4 | 8 | 3 | | |
| | 肺 | 細気管支-肺胞上皮腺腫 | 3 | 1 | 3 | 1 | | |
| | 口腔 | 扁平上皮乳頭腫 | 0 | 0 | 0 | 2 | | |
| | 下垂体 | 腺腫 | 19 | 16 | 14 | 11 | | |
| | 甲状腺 | C－細胞腺腫 | 7 | 9a) | 3a) | 7a) | | |
| | 膵島 | 島細胞腺腫 | 6 | 5 | 4 | 3 | | |
| | 副腎 | 褐色細胞腫 | 8 | 4 | 9 | 7 | | |
| | 精巣 | 間細胞腫 | 42 | 40 | 44 | 47 | | |
| 悪性腫瘍 | 脾臓 | 単核球性白血病 | 3 | 5 | 7a) | 4 | | |
| | 口腔 | 扁平上皮癌 | 0 | 0 | 0 | 5* | ↑↑ | ↑ |

酢酸ビニルのがん原性試験における主な腫瘍発生（ラット：雌）

| | 投与濃度（ppm） | | 0 | 400 | 2000 | 10000 | ペトー検定 | コクランアミテージ検定 |
|---|---|---|---|---|---|---|---|---|
| | 検査動物数 | | 50 | 50 | 50 | 50 | | |
| 良性腫瘍 | 下垂体 | 腺腫 | 8 | 11 | 9 | 14 | | |
| | 甲状腺 | C－細胞腺腫 | 2 | 7 | 8 | 5 | | |
| | 膵島 | 島細胞腺腫 | 0 | 1 | 3 | 1 | | |
| | 副腎 | 褐色細胞腫 | 4 | 1 | 1 | 2 | | |
| | 子宮 | 子宮内膜間質性ポリープ | 5 | 5 | 10 | 4 | | |
| | 乳腺 | 腺腫 | 1 | 3 | 0 | 1 | | |
| | | 線維腺腫 | 9 | 10 | 8 | 9 | | |
| | 陰核腺 | 腺腫 | 2 | 0 | 3 | 1 | | |
| 悪性腫瘍 | 脾臓 | 単核球性白血病 | 4 | 5 | 8 | 7 | | |
| | 口腔 | 扁平上皮癌 | 0 | 1 | 1 | 3 | ↑ | |
| | 食道 | 扁平上皮癌 | 0 | 0 | 0 | 1 | | |
| | 乳腺 | 腺癌 | 0 | 0 | 0 | 3 | | |

検定結果については生物学的意義を考慮して記載した。

＊：有意水準5%以下で有意　　＊＊：有意水準1%以下で有意（フィッシャー検定）

↑：有意水準5%以下で有意増加　↑↑：有意水準1%以下で有意増加（ペトー、コクランアミテージ検定）

↓：有意水準5%以下で有意減少　↓↓：有意水準1%以下で有意減少（コクランアミテージ検定）

a)：検査動物数49　他は上段に表示の検査動物数と同じ

酢酸ビニルのがん原性試験における主な腫瘍発生（マウス：雄）

| | 投与濃度（ppm） | | 0 | 400 | 2000 | 10000 | ペトー検定 | コクランアミテージ検定 |
|---|---|---|---|---|---|---|---|---|
| | 検査動物数 | | 50 | 50 | 50 | 50 | | |
| 良性腫瘍 | 肺 | 細気管支-肺胞上皮腺腫 | 3 | 3 | 4 | 3 | | |
| | 口腔 | 扁平上皮乳頭腫 | 0 | 0 | 0 | 4 | ↑↑ | ↑↑ |
| | 胃 | 扁平上皮乳頭腫 | 0 | 0 | 0 | 2 | | |
| | 肝臓 | 肝細胞腺腫 | 2 | 6 | 4 | 3 | | |
| | ハーダー腺 | 腺腫 | 3 | 4 | 6 | 2 | | |
| | 全臓器 | 血管腫 | 3 | 0 | 2 | 0 | | |
| 悪性腫瘍 | 喉頭 | 扁平上皮癌 | 0 | 0 | 0 | 2 | | |
| | 肺 | 細気管支-肺胞上皮腺癌 | 7 | 3 | 5 | 2 | | |
| | リンパ節 | 悪性リンパ腫 | 5 | 6 | 6 | 3 | | |
| | 脾臓 | 血管肉腫 | 2 | 1 | 4 | 0 | | |
| | 口腔 | 扁平上皮癌 | 0 | 0 | 0 | 13** | ↑↑ | ↑↑ |
| | 食道 | 扁平上皮癌 | 0 | 0 | 0 | 7* | ↑↑ | ↑↑ |
| | 胃 | 扁平上皮癌 | 1 | 0 | 0 | 7* | ↑↑ | ↑↑ |
| | 肝臓 | 血管肉腫 | 4 | 5 | 5 | 4 | | |
| | | 肝細胞癌 | 13 | 10 | 9 | 4* | | ↓ |
| | 全臓器 | 悪性リンパ腫 | 6 | 7 | 8 | 3 | | |
| | | 血管肉腫 | 6 | 7 | 7 | 4 | | |

酢酸ビニルのがん原性試験における主な腫瘍発生（マウス：雌）

| | 投与濃度（ppm） | | 0 | 400 | 2000 | 10000 | ペトー検定 | コクランアミテージ検定 |
|---|---|---|---|---|---|---|---|---|
| | 検査動物数 | | 50 | 50 | 50 | 49 | | |
| 良性腫瘍 | 肺 | 細気管支-肺胞上皮腺腫 | 1 | 3 | 1 | 2 | | |
| | 口腔 | 扁平上皮乳頭腫 | 0 | 0 | 0 | 3 | ↑↑ | ↑↑ |
| | 食道 | 扁平上皮乳頭腫 | 0 | 0 | 1 | 0 | | |
| | 胃 | 扁平上皮乳頭腫 | 0 | 0 | 0 | 1 | | |
| | 肝臓 | 肝細胞腺腫 | 3 | 1 | 4 | 0 | | |
| | 下垂体 | 腺腫 | 10 | 6[a] | 8[a] | 7 | | |
| | ハーダー腺 | 腺腫 | 0 | 4 | 3 | 0 | | |
| 悪性腫瘍 | 喉頭 | 扁平上皮癌 | 0 | 0 | 1 | 1 | | |
| | 肺 | 細気管支-肺胞上皮癌 | 2 | 3 | 1 | 1 | | |
| | リンパ節 | 悪性リンパ腫 | 11 | 10 | 17 | 10 | | |
| | 脾臓 | 悪性リンパ腫 | 0 | 5* | 1 | 1 | | |
| | 口腔 | 扁平上皮癌 | 0 | 0 | 0 | 15** | ↑↑ | ↑↑ |
| | 食道 | 扁平上皮癌 | 0 | 0 | 0 | 1 | | |
| | 胃 | 扁平上皮癌 | 0 | 0 | 0 | 3 | ↑↑ | ↑↑ |
| | 肝臓 | 血管肉腫 | 3 | 3 | 0 | 1 | | |
| | 子宮 | 組織球性肉腫 | 10 | 11 | 8 | 10[b] | | |
| | 全臓器 | 悪性リンパ腫 | 11 | 16 | 18 | 11 | | |
| | | 血管肉腫 | 4 | 5 | 1 | 1 | | |

検定結果については生物学的意義を考慮して記載した。

*：有意水準5%以下で有意　　**：有意水準1%以下で有意（フィッシャー検定）

↑：有意水準5%以下で有意増加　↑↑：有意水準1%以下で有意増加（ペトー、コクランアミテージ検定）

↓：有意水準5%以下で有意減少　↓↓：有意水準1%以下で有意減少（コクランアミテージ検定）

a)：検査動物数49　他は上段に表示の検査動物数と同じ

b)：検査動物数48　他は上段に表示の検査動物数と同じ

TABLE 1 EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE DRINKING WATER STUDIES OF VINYL ACETATE

Two-year studies

<Method of Administration>
Drinking water
<Number of Groups>
Male 4, Female 4
<Size of Groups>
50 males and 50 females of each group
<Animals>
Strain and Species
F344/DuCrj(Fischer) rat
Crj:BDF1 mouse
Animal Source
Charles River Japan, Inc.
Duration Held Before Study
2 wk
Age When Placed on Study
6 wk
Age When Killed
110～111 wk
<Doses>
Rat------<Male> 0, 400, 2000 or 10000 ppm
<Female> 0, 400, 2000 or 10000 ppm
Mouse---<Male> 0, 400, 2000 or 10000 ppm
<Female> 0, 400, 2000 or 10000 ppm
<Duration of Dosing>
7d/wk for 104wk
<Animal Maintenance>
Feed
CRF-1 (Oriental Yeast Co., Ltd.)
Sterilized by γ -ray
Available *ad libitum*
Water
Filtrated and sterilized by ultraviolet ray
Automatic watering system in duration of quarantine
Glass bottle in duration of acclimation and administration
Available *ad libitum*
Animal per Cage
Single (stainless steel wire)
Animal Room Environment
Barrier system
Temperature : 24±2℃
Humidity : 55±10%
Fluorescent light 12h/d
15-17 room air changes /h
<Type and Frequency of Observation>
Clinical Sign
Observed 1 per d
Body Weight
Weighed 1 per wk for 14wk
Weighed 1 per 2wks thereafter
Food Consumption
Weighed 1 per wk for 14wk
Weighed 1 per 4wks thereafter
Water Consumption
Weighed 2 per wk for 14wk
Weighed 1 per 2wks thereafter

TABLE 1 EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE DRINKING WATER STUDIES OF VINYL ACETATE (Continued)

| Two-year studies |
|---|
| <Hematology><br>Red blood cell (RBC),<br>Hemoglobin, Hematocrit,<br>Mean corpuscular volume (MCV),<br>Mean corpuscular hemoglobin (MCH),<br>Mean corpuscular hemoglobin concentration (MCHC),<br>Platelet, White blood cell (WBC),<br>Differential WBC,<br><Biochemistry><br>Total protein, Albumin,<br>A/G ratio, T-bilirubin, Glucose,<br>T-cholesterol, Triglyceride,<br>Phospholipid <rat only>,<br>Glutamic oxaloacetic transaminase (GOT),<br>Glutamic pyruvic transaminase (GPT),<br>Lactate dehydrogenase (LDH),<br>Alkaline phosphatase (ALP),<br>γ -Glutamyl transpeptidase (G-GTP) <rat only>,<br>Creatine phosphokinase (CPK), Urea nitrogen,<br>Creatinine <rat only>,<br>Sodium, Potassium, Chloride,<br>Calcium, Inorganic phosphorus,<br><Urinalysis><br>pH, Protein, Glucose, Ketone body<br>Bilirubin <rat only>, Occult blood<br>Urobilinogen,<br><Necropsy><br>Necropsy performed on all animals,<br><Organ weight><br>Organ weight measurement performed on scheduled sacrificed animals,<br>The following organs were weighed:<br>brain, lung, liver, spleen, heart, kidney, adrenal, testis, ovary,<br><Histopathologic Examination><br>Histopathologic examination performed on all animals,<br>The following organs were examined:<br>skin, nasal cavity, trachea,<br>lung, bone marrow, lymph node,<br>thymus, spleen, heart, tongue,<br>salivary gland, esophagus, stomach,<br>small intestine, large intestine, liver,<br>pancreas, kidney, urinary bladder,<br>pituitary, thyroid, adrenal, testis,<br>epididymis, seminal vesicle, prostate,<br>ovary, uterus, vagina, mammary gland,<br>brain, spinal cord, peripheral nerve,<br>eye, Harderian gland, muscle, bone, |

II－1－2　投与濃度

本がん原性試験の投与濃度は予備試験（2週間及び13週間試験）の結果に基づき決定した。

ラット、マウス雌雄とも最高用量を 10000ppmに設定し、それ以下、2000ppm、400ppm、(公比5.0)とした。なお、対照群として被験物質の希釈調製に用いた脱イオン水のみの群を設けた。

II－1－3　被験物質の調製方法

市水を脱イオンし、紫外線滅菌し、フィルターろ過した飲料水に被験物質を混合して各設定濃度になるように希釈調製した。なお、各試験における濃度の表示は、ppm（重量対重量比）とした。また、調製頻度は給水瓶交換に合わせて毎週2回とした。

II－1－4　調製時における濃度測定

各投与濃度に調製された飲水の被験物質濃度は、ガスクロマトグラフを用いて測定した。

各群の調製濃度は設定濃度に対し、ラットの試験では 400ppm群で81.0～120.8%、2000ppmで群89.0～102.7%、10000ppm群で71.4～112.0% 、マウスの試験では 400ppm群で81.0～113.3%、2000ppm群で88.4～118.4%、10000ppm群で74.1～120.0% の範囲にあった。

II－1－5　投与条件下における被験物質の安定性

被験物質の飲水中における安定性は、動物への投与前後（4日間）の調製被験物質の濃度をガスクロマトグラフを用いて分析し、それらの結果を比較することにより確認した。

4日目の測定濃度は設定濃度に対し、ラットの 400ppm群で80%、2000ppmで群72%、10000ppm群で74% 、マウスの試験では 400ppm群で94%、2000ppm群で96%、10000ppm群で86%であった。

II－1－6　被験物質の摂取量

各計測週内の後半4日間における摂水量より被験物質の体重当たりの摂取量(g/kg/day)を算出した。

II－2　動物管理

II－2－1　各群の使用動物数

ラット、マウスとも投与群3群及び対照群1群の計4群を設け、雌雄各群50匹とし、それぞれ計400匹の動物を用いた。

II－2－2　群分け及び個体識別方法

供試動物の各投与群への割り当ては、適正層別方式すなわち動物を体重の重い順より各群に1匹づつ割り当て、二巡目からは各群の動物の体重の合計を比較して小さい群より順に体重の重い動物を割り当てることにより群間の体重の偏りを小さくする群分け方法により実施した(文献 4)。

試験期間中の動物の個体識別は、検疫期間及び馴化期間においては色素塗布により、投与期間においては耳パンチにより識別し、またケージにも個体識別番号を付した。

なお、ラットとマウスは、バリア区域内の独立した室にそれぞれ収容し、各室に試験番号、動物種及び動物番号を表示し、他試験及び異種動物と区別した。

II－2－3　飼育条件

動物は、各試験ともに全飼育期間を通して、温度24±2℃、湿度55±10%、明暗サイクル:12時間点灯(8:00～20:00)/12時間消灯(20:00～8:00)、換気回数15～17回/時の環境下で飼育した。

動物は単飼ケージ(ステンレス製二連網ケージ、ラット:170W×294D×176H mm、マウス:112W×212D×120H mm)に収容し、ケージ交換は2週間毎に実施した。

飼料は、オリエンタル酵母工業(株)のCRF-1固型飼料(3Mrad-γ線照射滅菌飼料)を使用し、全飼育期間を通して固型飼料給餌器により自由摂取させた。

飲水は、市水(桑野市水道局供給)をフィルターろ過した後、紫外線滅菌し、検疫期間については自動給水装置で、馴化期間及び投与期間は褐色のガラス製給水瓶によって自由摂取させた。なお、給水瓶交換は週2回行った。

なお、使用飼料の品質管理は栄養成分についてはオリエンタル酵母工業(株)の自社分析データ資料を、夾雑物については(財)日本食品分析センター、飲水の品質については(財)食品薬品安全性センターの分析データ資料を使用ロット毎に入手し異常のないことを確認した。

II－3　観察・検査項目及び方法

II－3－1　動物の一般状態の観察

各試験とも、毎日1回、動物の一般状態の観察を行った。

II－3－2　体重測定

投与開始後14週までは週1回、それ以降は2週に1回、体重を測定した。
なお、動物の死亡発見時及び切迫屠殺時も体重を測定した。

II－3－3　摂水量測定

投与開始後14週までは週2回、それ以降は2週に1回（週内後半4日間）、摂水量を個体別に測定した。

II－３－４　摂餌量測定

投与開始後14週までは週1回、それ以降は4週に1回、摂餌量を個体別に測定した。

II－３－５　血液学的検査

定期解剖時まで生存した動物について、剖検直前にエーテル麻酔下で腹大動脈よりEDTA-2K入り採血管に採血した血液を用いて血液学的検査を行った。なお、検査対象動物は解剖日前日より18時間以上絶食させた。検査項目は TABLE 1 に示した。

II－３－６　血液生化学的検査

定期解剖時まで生存した動物について、剖検直前にエーテル麻酔下で腹大動脈よりヘパリンリチウム入り採血管に採血した血液を遠心分離して得られた血漿を用いて血液生化学的検査を行った。なお、検査対象動物は解剖日前日より18時間以上絶食させた。検査項目は TABLE 1 に示した。

II－３－７　尿検査

投与最終週まで生存した動物について、新鮮尿を採取し、尿検査を行った。検査項目は TABLE 1 に示した。

II－３－８　病理学的検査

（１）剖検
全ての動物について剖検を行った。

（２）臓器重量

定期解剖時まで生存した動物について TABLE 1 に示した臓器の実重量を測定した。また、実重量の体重比、すなわち定期解剖時の体重に対する百分率を算出した。

（３）病理組織学的検査

全動物の臓器を10%中性リン酸緩衝ホルマリン溶液にて固定後、TABLE 1に示した臓器及び肉眼的に変化のみられた組織を、パラフィン包埋、薄切、ヘマトキシリン・エオジン染色し、光学顕微鏡にて病理組織学的に検査した。なお、試験期間後期(ラット97週、マウス94週以降)に解剖した動物はTABLE 1に示した臓器に加え、下顎を保存し標本作製を行った。

## II－４　数値処理と統計学的方法

### II－４－１　数値の取り扱いと表示

体重についてはｇを単位とし、ラットでは小数点以下第1位を四捨五入して整数値で、マウスでは小数点以下第2位を四捨五入して小数点以下第1位までを表示した。

摂餌量についてはｇを単位とし、計測期間を通しての摂餌量を小数点以下第1位まで計測し、この値を計測期間の日数で除し、1日当たりの平均摂餌量を算出し、小数点以下第2位を四捨五入して小数点以下第1位までを表示した。

摂水量についてはｇを単位とし、計測期間を通しての摂水量を小数点以下第1位まで計測し、この値を計測期間の日数で除し、1日当たりの平均摂水量を算出し、小数点以下第2位を四捨五入して小数点以下第1位までを表示した。

酢酸ビニルの体重当りの摂取量は摂水量に酢酸ビニルの設定濃度を乗じ体重で除した値を g/kg(body weight)/dayを単位として小数点以下第4位を四捨五入して小数点以下第3位まで表示した。

臓器実重量についてはｇを単位とし、小数点以下第3位まで計測し、表示した。臓器重量体重比については臓器実重量値を解剖時体重で除し、パーセント単位で小数点以下第4位を四捨五入し、小数点以下第3位までを表示した。

A/G比はアルブミン/(総蛋白－アルブミン)による計算で求め、小数点以下第2位を四捨五入して小数点以下第1位までを表示した。

なお、各数値データにおいての平均値及び標準偏差は上記に示した桁数と同様になるよう四捨五入を行い表示した。

### II－４－２　母数の取り扱い

体重及び摂餌量については、各計測時に生存している全動物を対象に計測し、欠測となったデータについては母数より除いた。

臓器重量、血液学的検査、血液生化学的検査は、定期解剖時まで生存した動物を対象とし、欠測となったデータについては母数より除いた。

尿検査は、投与最終週まで生存した動物を対象に行い、検査数を母数とした。

剖検と病理組織学的検査データは、各群の有効動物数(供試動物より事故等の理由で外された動物を減じた動物数)を母数とした。ただし、腫瘍性病変については臓器別に、検査不能臓器数を除いたものを母数とした。

### II－４－３　統計学的方法

本試験で得られた測定値は対照群を基準群として、まずBartlett法により等分散の予備検定を行い、その結果が等分散の場合には一元配置分散分析を行い、群間に有意差が認められた場合はDunnettの多重比較により平均値の検定を行った。また、分散の等しくない

場合には各群を通して測定値を順位化して、Kruskal-Wallisの順位検定を行い、群間に有意差が認められた場合にはDunnett(型)の多重比較を行った。

予備検定については5%の有意水準で両側検定を行い、最終検定では5%及び1%で両側検定を行った。

なお、病理組織学的検査のうち非腫瘍性病変については、所見のみられなかった動物をグレード0として$\chi^2$検定を行った。また、尿検査についても$\chi^2$検定を行った。

腫瘍性病変については、各臓器腫瘍毎に、各群毎の総担癌臓器数について、Peto検定（文献 5）、Cochran-Armitage検定、Fisher検定を行った。またPeto検定は病理組織学的検査時に付与されたコンテックス（注）を用いて、死亡率法（コンテックス3,4を付与された腫瘍についての検定）、有病率法（コンテックス0,1,2を付与された腫瘍についての検定）、死亡率法＋有病率法（コンテックス0～4の総計で検定）でそれぞれ検定を行った。

$\chi^2$検定とFisher検定は対照群と各投与群間との検定である。

各群雌雄毎に検査数が２以下の項目については検定より除外した。

注： Peto検定に用いるコンテックス

0：定期解剖例にみつかった腫瘍

1：死亡／瀕死例にみつかった腫瘍で、直接死因に関係しない腫瘍

2：多分1だと思うが、確かでない腫瘍

3：多分4だと思うが、確かでない腫瘍

4：死亡／瀕死例にみつかった腫瘍で、直接死因に関わっていた腫瘍

## Ⅲ　試験成績

### Ⅲ－１　ラットを用いたがん原性試験

#### Ⅲ－１－１　動物の状態観察

（１）生死状況

生死状況を FIGURE 1,2 に示した。

投与群の生存率は雌雄とも対照群と比べ顕著な差は認められなかった。

各群の104週における生存動物数(生存率)は、雄では対照群:44/50例（88%)、400ppm群:40/50例(80%)、2000ppm群:36/50例(72%)、10000ppm群:39/50例(78%)、雌では対照群:41/50例(82%)、400ppm群:40/50例(80%)、2000ppm群:41/50例(82%)、10000ppm群:37/50例(74%)であった。

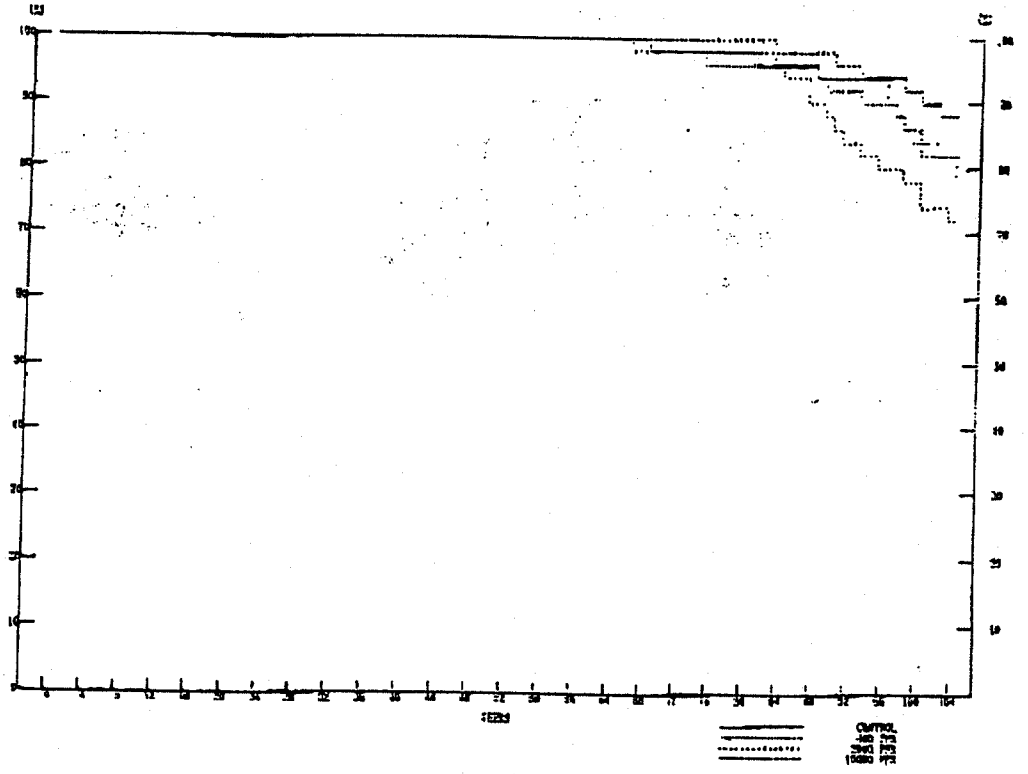

FIGURE 1　SURVIVAL ANIMAL RATE (RAT:MALE)

（２）一般状態

一般状態の観察結果のうち外部腫瘤、内部腫瘤の発生動物数をTABLE 2､3 に示した。

酢酸ビニル投与の影響とみられる口腔の腫瘤が雄では 2000ppm群の1例と10000ppm群の2例に、雌では 400ppm群と 10000ppm群の各1例に観察された。　その他の外部腫瘤や内部腫瘤の発生には雌雄ともに各投与群と対照群の間に顕著な差は認められなかった。その他の一般状態では、酢酸ビニル投与による特徴的な所見を死亡／瀕死例、定期解剖例のいずれにも認めなかった。

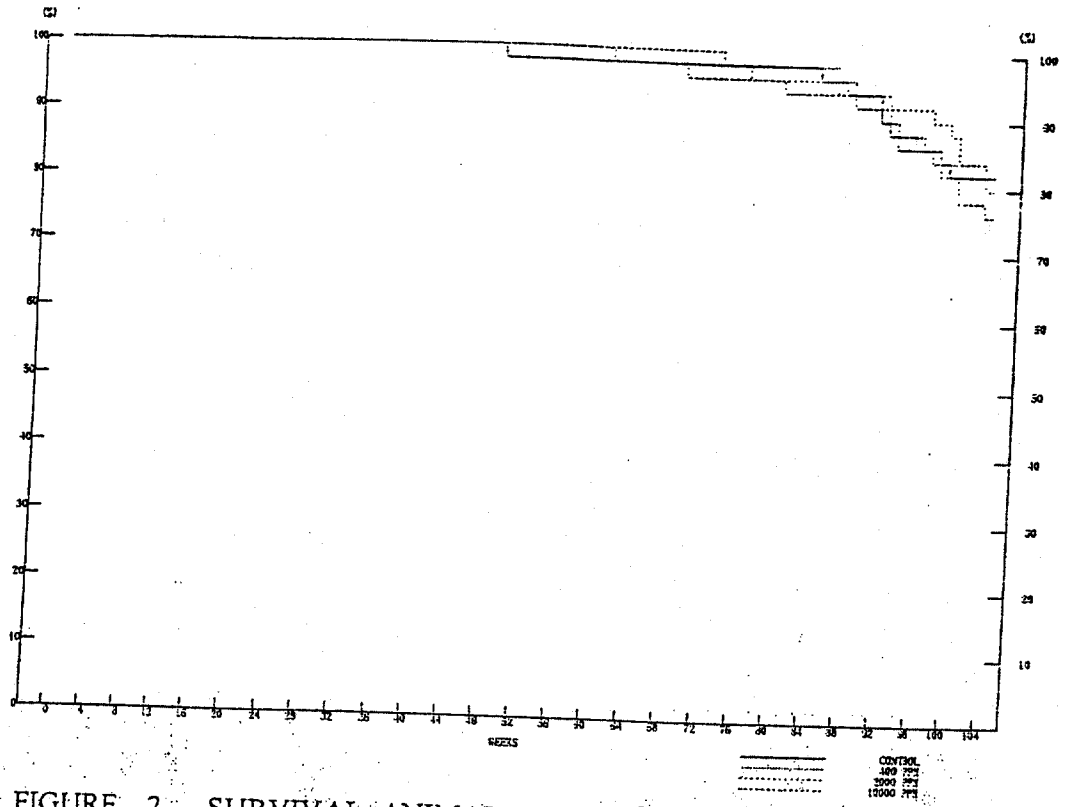

FIGURE 2 SURVIVAL ANIMAL RATE (RAT:FEMALE)

TABLE 2 INCIDENCE OF EXTERNAL AND INTERNAL MASS IN CLINICAL OBSERVATION IN MALE RATS

| Time of mass occurrence (week) | 0～13 | 14～26 | 27～39 | 40～52 | 53～65 | 66～78 | 79～91 | 92～104 | 0～104 |
|---|---|---|---|---|---|---|---|---|---|
| External mass (Includeing Mass[Oral cavity]) | | | | | | | | | |
| Control | 0/50 | 0/50 | 0/50 | 0/50 | 2/50 | 3/50 | 7/49 | 11/47 | 13/50 (2/ 6) |
| 400 ppm | 0/50 | 1/50 | 1/50 | 1/50 | 2/50 | 5/50 | 7/48 | 14/46 | 16/50 (4/10) |
| 2000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 4/49 | 10/49 | 21/42 | 23/50 (5/14) |
| 10000 ppm | 0/50 | 0/50 | 1/50 | 1/50 | 1/50 | 2/50 | 4/50 | 9/48 | 10/50 (2/11) |
| Mass [Oral cavity] | | | | | | | | | |
| Control | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/49 | 0/47 | 0/50 (0/ 6) |
| 400 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/48 | 0/46 | 0/50 (0/10) |
| 2000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/49 | 0/49 | 0/42 | 0/50 (0/14) |
| 10000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 2/48 | 2/50 (0/11) |
| Internal mass | | | | | | | | | |
| Control | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/49 | 0/47 | 0/50 (0/ 6) |
| 400 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 1/50 | 1/48 | 0/46 | 2/50 (1/10) |
| 2000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/49 | 1/49 | 2/42 | 3/50 (2/14) |
| 10000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 1/48 | 1/50 (1/11) |

No. of animals with mass/No. of survival animals at first week on each period.
(No. of dead and moribund animals with mass/No. of dead and moribund animals.)

TABLE 3 INCIDENCE OF EXTERNAL AND INTERNAL MASS IN CLINICAL OBSERVATION IN FEMALE RATS

| Time of mass occurrence (week) | 0～13 | 14～26 | 27～39 | 40～52 | 53～65 | 66～78 | 79～91 | 92～104 | 0～104 |
|---|---|---|---|---|---|---|---|---|---|
| External mass (Including Mass[Oral cavity]) | | | | | | | | | |
| Control | 0/50 | 1/50 | 1/50 | 1/50 | 1/49 | 3/49 | 6/49 | 10/44 | 11/50 (3/ 9) |
| 400 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 1/50 | 2/50 | 6/49 | 8/44 | 9/50 (2/10) |
| 2000 ppm | 0/50 | 0/50 | 0/50 | 1/50 | 2/50 | 3/50 | 5/48 | 10/46 | 13/50 (3/ 9) |
| 10000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 2/50 | 3/49 | 4/48 | 9/45 | 11/50 (3/13) |
| Mass [Oral cavity] | | | | | | | | | |
| Control | 0/50 | 0/50 | 0/50 | 0/50 | 0/49 | 0/49 | 0/49 | 0/44 | 0/50 (0/ 9) |
| 400 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 1/49 | 1/44 | 1/50 (0/10) |
| 2000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/48 | 0/46 | 0/50 (0/ 9) |
| 10000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/49 | 0/48 | 1/45 | 1/50 (1/13) |
| Internal mass | | | | | | | | | |
| Control | 0/50 | 0/50 | 0/50 | 0/50 | 0/49 | 0/49 | 1/49 | 2/44 | 3/50 (1/ 9) |
| 400 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 1/49 | 0/44 | 1/50 (1/10) |
| 2000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 1/50 | 1/48 | 1/46 | 3/50 (3/ 9) |
| 10000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/49 | 0/48 | 6/45 | 6/50 (4/13) |

No. of animals with mass/No. of survival animals at first week on each period.
(No. of dead and moribund animals with mass/No. of dead and moribund animals.)

（3）体重

体重の推移を FIGURE 3,4 に示した。

雌雄ともに最高用量の10000ppm群でわずかに体重増加の抑制が認められ、その抑制率は対照群に比べ最大で雄8%、雌10%であった。

（4）摂水量

摂水量を FIGURE 5,6 に示した。

雌雄ともに最高用量の 10000ppm群に摂水量の低下が認められた。10000ppm群の全投与期間中の平均摂水量は対照群に対し、雄では 83%、雌では 75%であった。

（5）摂餌量

摂餌量を FIGURE 7,8 に示した。

雌雄ともに投与群の摂餌量は、対照群と比較して顕著な差は認められなかった。

（6）被験物質摂取量

体重、摂水量及び設定濃度より被験物質摂取量(g/kg/day)を算出したた。

被験物質の1日当たりの摂取量は、雄で 400ppm群：0.016～0.048g/kg、2000ppm群：0.075～0.226g/kg、10000ppm群：0.364～0.950g/kg、雌では 400ppm群：0.022～0.060 g/kg、2000ppm群：0.109～0.266g/kg、10000ppm群：0.478～1.062g/kgであった。

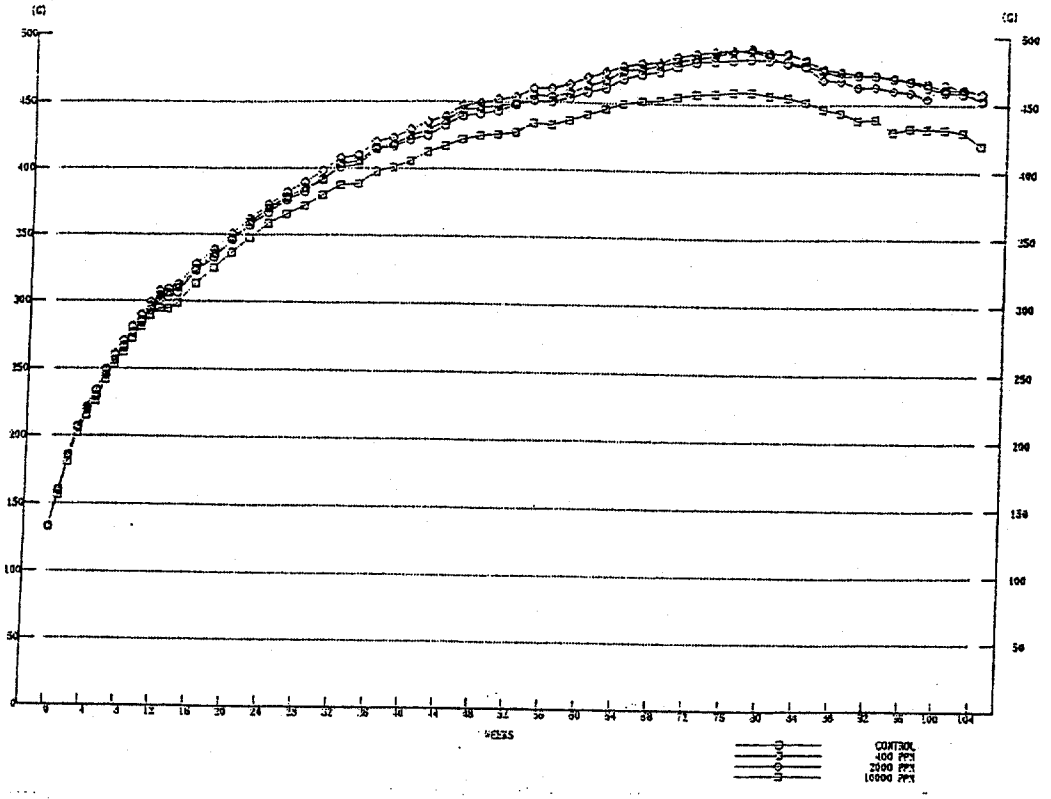

FIGURE 3 BODY WEIGHT CHANGES (RAT:MALE)

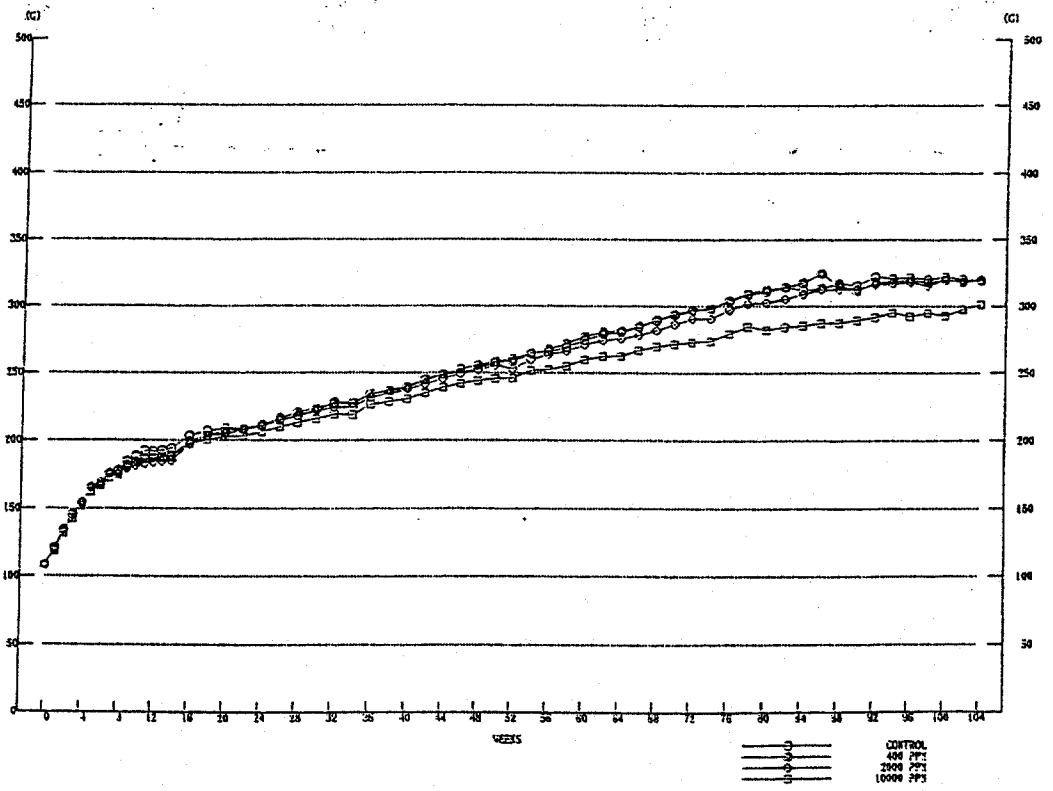

FIGURE 4 BODY WEIGHT CHANGES (RAT:FEMALE)

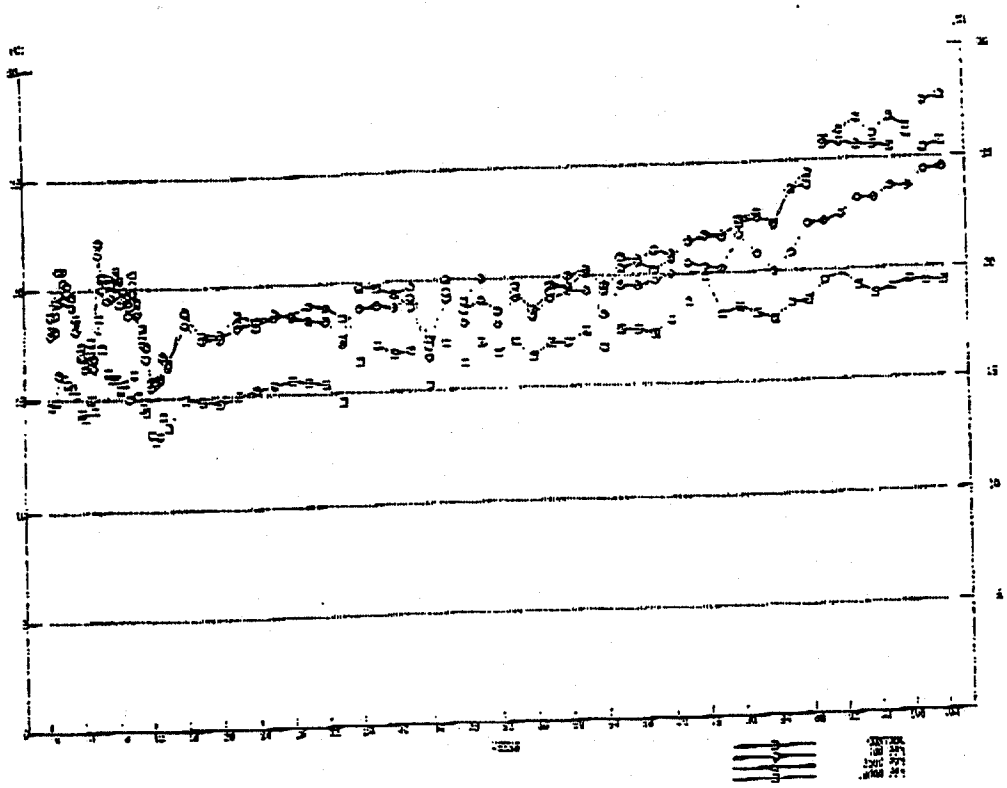

FIGURE 5 WATER CONSUMPTION CHANGES (RAT:MALE)

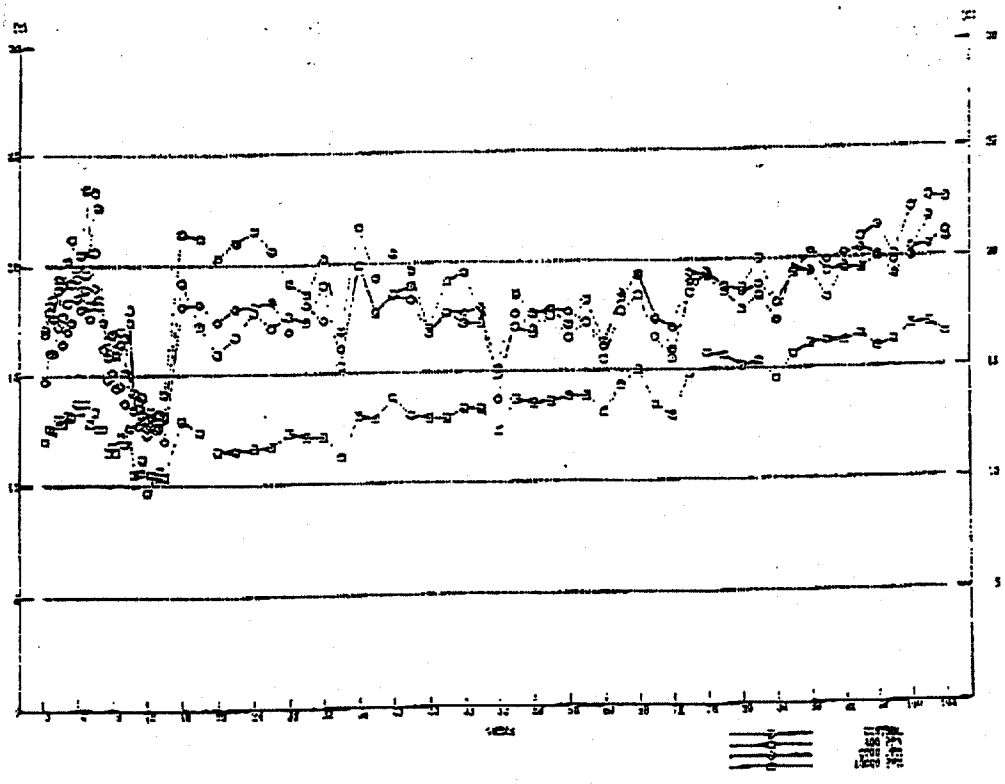

FIGURE 6 WATER CONSUMPTION CHANGES (RAT:FEMALE)

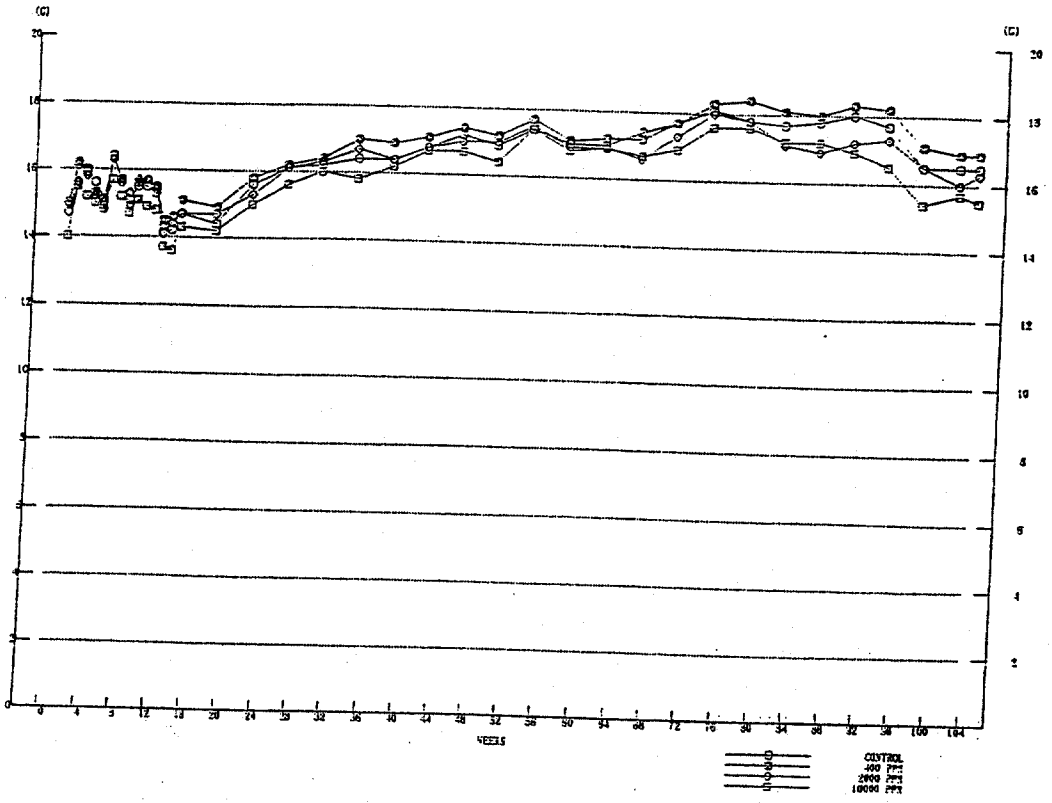

FIGURE 7 FOOD CONSUMPTION CHANGES (RAT:MALE)

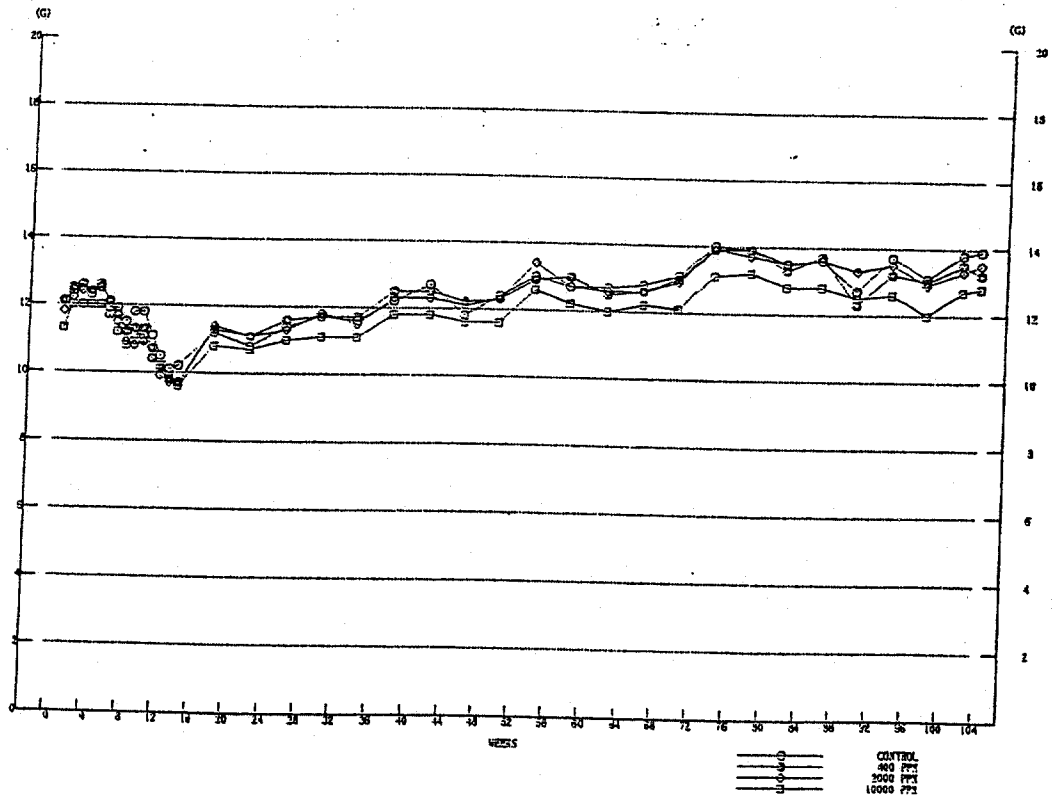

FIGURE 8 FOOD CONSUMPTION CHANGES (RAT:FEMALE)

Ⅲ－１－２　血液学的検査・血液生化学的検査

（１）血液学的検査

雄では、対照群に比べて著変は認められなかった。

雌では、400ppm群でヘモグロビン濃度とＭＣＨの増加、2000ppm群でＭＣＨＣの増加が認められたが、これらはごく軽度な変化であり投与量に相関したものではなかった。

（２）血液生化学的検査

雄では、最高用量の 10000ppm群でＡ／Ｇ比の増加、並びに総コレステロール、リン脂質及びカルシウムの減少が認められた。

雌では、対照群に比べて著変は認められなかった。

（３）尿検査

雄では、400ppm群と 10000ppm群でｐＨの低下が、10000ppm群でケトン体の疑陽性例の増加が認められた。

雌では、対照群に比べて著変は認められなかった。

Ⅲ－１－３　病理学的検査

（１）剖検

下顎の結節が、雄では 10000ppm群の3/50例に、雌では 10000ppm群の1/50例と 400ppm群の1/50例に観察された。また、雄の腎臓の顆粒状変化の発生が投与濃度に対応して低下した(対照群:16/50例、400ppm群:18/50例、2000ppm群:11/50例、10000ppm群:6/50例)。

（２）臓器重量

雄の 10000ppm群に腎臓と肝臓の実重量の低値が認められたが、これは解剖時の体重の低値に伴う変化と考えられた。

（３）病理組織学的検査

主な腫瘍性病変とそれに関連した非腫瘍性病変を TABLE 4,5 に示した。

－主な腫瘍性病変－

(口腔)

雄では扁平上皮癌の発生(対照群:0/50例、400ppm群:0/50例、2000ppm群:0/50例、10000ppm群:5/50例)がPeto検定(死亡率法、有病率法、死亡率法+有病率法)とCochran-Armitage検定で増加傾向を示し、Fisher検定でも 10000ppm群に対照群と比べて増加が認められた。また、扁平上皮乳頭腫が10000ppm群の2/50例に認められた。扁平上皮乳頭腫と扁平上皮癌を合わせた発生(対照群:0/50例、400ppm群:0/50例、2000ppm群:0/50例、10000ppm群:7/50例)も、Peto検定(死亡率法、有病率法、死亡率法＋有病率法)とCochran-Armitage検定で増加傾向を示し、Fisher検定でも 10000ppm群は対照群と比べて増加を示した。雌でも扁平上皮癌の発生(対照群:0/50例、400ppm群:1/50例、2000ppm群:1/50例、10000ppm群:3/50例)がPeto検定(有病率法)で増加傾向を示した(TABLE 4,5,6,7)。

なお、扁平上皮癌の舌への転移が雄 10000ppm群の1/50例に認められた。

Table 4 SELECTED LESIONS OF DIGESTIVE SYSTEM IN MALE RATS

| | neoplastic disease | | | | | | | | non neoplastic disease | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | squamous cell papilloma | | | | squamous cell carcinoma | | | | squamous cell hyperplasia | | | | basal cell activation | | | | epithlial dysprasia | | | |
| Group(ppm) | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 |
| Number of examinec | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| oral cavity | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| esophagus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| stomach | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |

Table 5 SELECTED LESIONS OF DIGESTIVE SYSTEM IN FEMALE RATS

| | neoplastic disease | | | | | | | | non neoplastic disease | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | squamous cell papilloma | | | | squamous cell carcinoma | | | | squamous cell hyperplasia | | | | basal cell activation | | | | epithlial dysprasia | | | |
| Group(ppm) | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 |
| Number of examinec | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| oral cavity | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| esophagus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 |
| stomach | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |

TABLE 6 NEOPLASTIC LESIONS (ORAL CAVITY) INCIDENCE AND STATISTICAL ANALYSIS IN MALE RATS

| Group Name | Control | 400 ppm | 2000 ppm | 10000 ppm |
|---|---|---|---|---|
| SITE : oral cavity | | | | |
| TUMOUR : squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 5/50 (10.0) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 7.69 |
| Terminal Rates(c) | 0/44 ( 0.0) | 0/40 ( 0.0) | 0/36 ( 0.0) | 3/39 ( 7.7) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.0161* ? | | | |
| Prevalence Method(d) | P=0.0019**? | | | |
| Combined analysis(d) | P=0.0001**? | | | |
| Cochran-Armitage Test(e) | P=0.0001** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.0360* |
| SITE : oral cavity | | | | |
| TUMOUR : squamous cell papilloma, squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 7/50 (14.0) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 12.82 |
| Terminal Rates(c) | 0/44 ( 0.0) | 0/40 ( 0.0) | 0/36 ( 0.0) | 5/39 (12.8) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.0161* ? | | | |
| Prevalence Method(d) | P<0.0001**? | | | |
| Combined analysis(d) | P<0.0001**? | | | |
| Cochran-Armitage Test(e) | P<0.0001**? | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.0101* |

TABLE 7 NEOPLASTIC LESIONS (ORAL CAVITY) INCIDENCE AND STATISTICAL ANALYSIS IN FEMALE RATS

| Group Name | Control | 400 ppm | 2000 ppm | 10000 ppm |
|---|---|---|---|---|
| SITE : oral cavity | | | | |
| TUMOUR : squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 1/50 ( 2.0) | 1/50 ( 2.0) | 3/50 ( 6.0) |
| Adjusted Rates(b) | 0.0 | 2.50 | 2.46 | 8.11 |
| Terminal Rates(c) | 0/41 ( 0.0) | 1/40 ( 2.5) | 1/41 ( 2.4) | 3/37 ( 8.1) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=------ | | | |
| Prevalence Method(d) | P=0.0342* | | | |
| Combined analysis(d) | P=------ | | | |
| Cochran-Armitage Test(e) | P=0.0590 | | | |
| Fisher Exact Test(e) | | P=0.4950 | P=0.4950 | P=0.1325 |

(a) :Number of tumor-bearing animals/number of animals examined at the site.
(b) :Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c) :Observed tumor incidence at terminal kill.
(d) :Beneth the control incidence are the P-values associated with the trend test.
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combined analysis : Death analysis + Incidental tumor test
(e) :The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates.
------: There is no data which should be statistical analysis.
Significant difference : * : P ≦ 0.05 ** : P ≦ 0.01

(食道)
扁平上皮癌が雌 10000ppm群の1/50例に認められた(TABLE 4,5)。

ーその他の腫瘍性病変ー
雄の精巣の間細胞腫の発生(対照群:42/50例、400ppm群:40/50例、2000ppm群:44/50例、10000ppm群:47/50例)がPeto検定(有病率法)で増加傾向を示したが、10000ppm群の発生率も当センターのヒストリカルコントロールデータ(文献 6)の範囲内(平均:89.6%,試験単位での発生率:82～98%)であることから、被験物質の投与による影響とは考えられなかった(TABLE 8)。

また、雌の乳腺の腺癌の発生(対照群:0/50例、400ppm群:0/50例、2000ppm群:0/50例、10000ppm群:3/50例)が、Peto検定(有病率、死亡率+有病率法)とCochran-Armitage検定で増加傾向を示した。しかし、この発生率もヒストリカルコントロールデータ(文献 6)の範囲内(平均:2.0%,試験単位での発生率:0～6%)であり、被験物質の投与による影響とは断定できなかった(TABLE 9)。

TABLE 8 NEOPLASTIC LESIONS (TESTIS) INCIDENCE AND STATISTICAL ANALYSIS IN MALE RATS

| Group Name | Control | 400 ppm | 2000 ppm | 10000 ppm |
|---|---|---|---|---|
| SITE : testis | | | | |
| TUMOUR : interstitial cell tumor | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 42/50 (84.0) | 40/50 (80.0) | 44/50 (88.0) | 47/50 (94.0) |
| Adjusted Rates(b) | 88.89 | 86.05 | 94.59 | 100.00 |
| Terminal Rates(c) | 39/44 (88.6) | 34/40 (85.0) | 34/36 (94.4) | 39/39(100.0) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard method(d) | P=------ | | | |
| Prevalence method(d) | P=0.0188* | | | |
| Combined analysis(d) | P=------ | | | |
| Cochran-Armitage Test(e) | P=0.0543 | | | |
| Fisher Exact Test(e) | | P=0.4942 | P=0.4956 | P=0.4053 |

TABLE 9 NEOPLASTIC LESIONS (MAMMARY GLAND) INCIDENCE AND STATISTICAL ANALYSIS IN FEMALE RATS

| Group Name | Control | 400 ppm | 2000 ppm | 10000 ppm |
|---|---|---|---|---|
| SITE : mammary gland | | | | |
| TUMOUR : adenocarcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 3/50 ( 6.0) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 5.41 |
| Terminal Rates(c) | 0/41 ( 0.0) | 0/40 ( 0.0) | 0/41 ( 0.0) | 2/37 ( 5.4) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.1723 | | | |
| Prevalence Method(d) | P=0.0117* ? | | | |
| Combined analysis(d) | P=0.0017**? | | | |
| Cochran-Armitage Test(e) | P=0.0030** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.1325 |

(a) :Number of tumor-bearing animals/number of animals examined at the site.
(b) :Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c) :Observed tumor incidence at terminal kill.
(d) :Beneth the control incidence are the P-values associated with the trend test.
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combined analysis : Death analysis + Incidental tumor test
(e) :The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates.
? :The conditional probabilities of the largest and smallest possible out comes can not estimated or this P-value is beyond the estimated P-value.
------: There is no data which should be statistical analysis.
Significant difference ; * : $P \leqq 0.05$ ** : $P \leqq 0.01$

さらに、雌の甲状腺のC-細胞腺腫とC-細胞癌を合わせた発生(対照群:2/50例、400ppm群:7/50例、2000ppm群:9/50例、10000ppm群:6/50例)が、Fisher検定で 2000ppm群と対照群の間に有意差が認められた(TABLE 10)。しかし、この変化も投与濃度に対応した傾向を示していないことから、被験物質の投与による影響とは考えられなかった。

TABLE 10 NEOPLASTIC LESIONS (THYROID) INCIDENCE AND STATISTICAL ANALYSIS IN FEMALE RATS

| Group Name | Control | 400 ppm | 2000 ppm | 10000 ppm |
|---|---|---|---|---|
| SITE : thyroid | | | | |
| TUMOUR : C-cell adenoma, C-cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 2/50 ( 4.0) | 7/50 (14.0) | 9/50 (18.0) | 6/50 (12.0) |
| Adjusted Rates(b) | 4.88 | 15.00 | 20.45 | 15.38 |
| Terminal Rates(c) | 2/41 ( 4.9) | 6/40 (15.0) | 8/41 (19.5) | 5/37 (13.5) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=------ | | | |
| Prevalence Method(d) | P=0.3422 | | | |
| Combined analysis(d) | P=------ | | | |
| Cochran-Armitage Test(e) | P=0.7312 | | | |
| Fisher Exact Test(e) | | P=0.1045 | P=0.0427* | P=0.1606 |

(a):Number of tumor-bearing animals/number of animals examined at the site.
(b):Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c):Observed tumor incidence at terminal kill.
(d):Beneth the control incidence are the P-values associated with the trend test.
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combined analysis : Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates.
------: There is no data which should be statistical analysis.
Significant difference : * : P ≦ 0.05 ** : P ≦ 0.01

ー非腫瘍性病変ー

(口腔)

基底細胞の賦活化が 10000ppm群の雄2/50例と雌1/50例に、また上皮の異形成が 10000ppm群の雌2/50例に認められた(TABLE 4,5)。

(食道)

基底細胞の賦活化が 10000ppm群の雌4/50例、扁平上皮過形成が 10000ppm群の雌雄各1/50例に認められた(TABLE 4,5)。

(胃)

前胃の基底細胞の賦活化が 10000ppm群の雄2/50例と雌5/50例に認められ、雌 10000ppm群の発生率は10%と対照群の0%と比較して統計学的にも有意に増加していた(TABLE 4,5)。

(腎臓)

定期解剖例でみた場合、慢性腎症の程度の低下が、雄 10000ppm群に認められた。

－その他の非腫瘍性病変－

鼻腔の嗅上皮のエオジン好性変化の程度の増強が雌の 10000ppm群の途中死亡／瀕死例に、また副腎の髄質細胞増生の発生低下が、雄の 2000ppm群と 10000ppm群の途中死亡／瀕死例にみられた。しかし、これらの所見は定期解剖例に認められないことから、被験物質投与との関連は明らかではなかった。また、雌の 10000ppm群の定期解剖例で肝臓の胆管増生の程度が弱まったが、被験物質投与との関連は明らかではなかった。上記の所見の他に、対照群との比較で統計学的に有意な差が認められた非腫瘍性病変、すなわち定期解剖例の雄の鼻腔の腺の呼吸上皮化生の低下、眼球の網膜萎縮の増加及び雌の肝臓の明細胞性小増殖巣の低下が認められた。しかし、これらの所見の発生率は投与濃度に対応したものではないことから、被験物質の投与による影響とは考えられなかった。

（4）死因

病理学的にみた死亡／瀕死の原因を TABLE 11 に示した。

雌雄とも各投与群と対照群の間に顕著な差を認めなかった。

TABLE 11 CAUSE OF DEATH IN RATS

| Group | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 400ppm | 2000ppm | 10000ppm | Control | 400ppm | 2000ppm | 10000ppm |
| Number of dead/moribund animal | 6 | 10 | 14 | 11 | 9 | 10 | 9 | 13 |
| No microscopical confirmation | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Cardiovascular lesion | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| Chronic nephropathy | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tumor death : leukemia | 1 | 3 | 3 | 3 | 2 | 2 | 3 | 4 |
| : skin/appendage | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| : subcutis | 1 | 2 | 2 | 2 | 2 | 0 | 0 | 0 |
| : larynx | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 |
| : bone marrow | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| : spleen | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| : oral cavity | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| : liver | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 1 |
| : pituitary gland | 2 | 3 | 3 | 1 | 1 | 6 | 1 | 3 |
| : uterus | – | – | – | – | 2 | 0 | 2 | 1 |
| : mammary gland | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 |
| : prep./cli. gland | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| : brain | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 |
| : Zymbal gland | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| : bone | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| : vertebra | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| : retroperitoneum | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |

## Ⅲ－2　マウスを用いたがん原性試験

### Ⅲ－2－1　動物の状態観察

（1）生死状況

生死状況を FIGURE 9,10 に示した。

投与群の生存率は雌雄とも対照群に比べ顕著な差は認められなかった。

各群の104週における生存動物数(生存率)は、雄では対照群:35/50例(70%)、400ppm群:42/50例(84%)、2000ppm群:38/50例(76%)、10000ppm群:33/50例(66%)、雌では対照群:26/50例(52%)、400ppm群:27/50例(54%)、2000ppm群:25/50例(50%)、10000ppm群:23/50例(46%)であった。

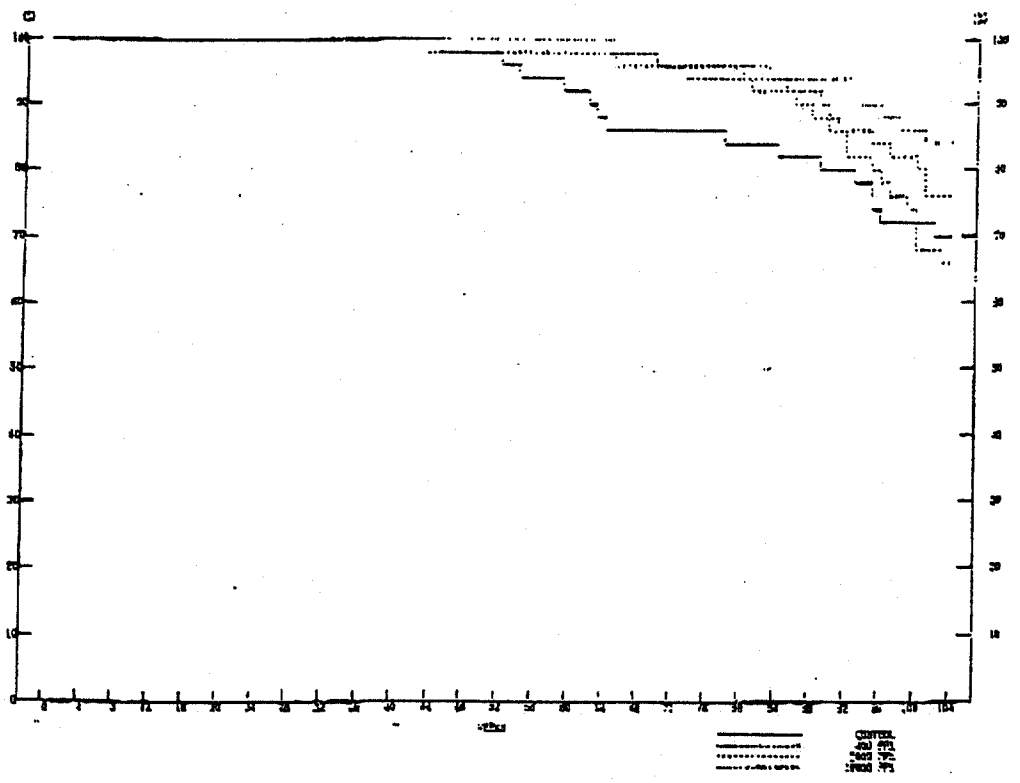

FIGURE 9　SURVIVAL ANIMAL RATE (MOUSE:MALE)

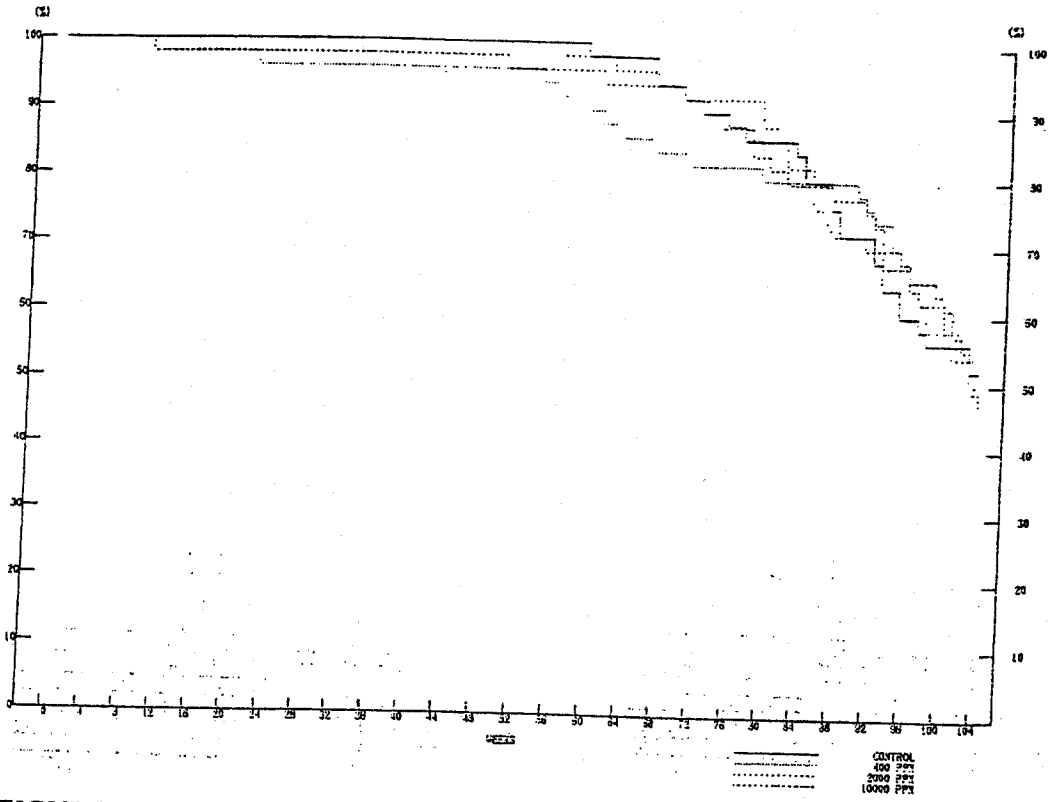

FIGURE 10 SURVIVAL ANIMAL RATE (MOUSE:FEMALE)

（２）一般状態

一般状態の観察結果のうち外部腫瘤、内部腫瘤の発生動物数を TABLE 12,13 に示した。

酢酸ビニルの投与の影響とみられる口腔の腫瘤が雌雄とも最高用量の 10000ppm群の各6/50例に観察された。その他の外部腫瘤の発生については投与群と対照群の間に顕著な差を認めなかった。内部腫瘤の発生状況を全動物(死亡／瀕死例、定期解剖例)についてみると、雄では、対照群は10/50例に対し、400ppm群では18/50例、10000ppm群では5/50例と発生数に差が認められたが、投与濃度に対応した変化ではなかった。雌では、内部腫瘤の発生状況に顕著な差は認められなかった。その他の一般状態では、酢酸ビニルによる特徴的な所見は死亡／瀕死例、定期解剖例のいずれにも認めなかった。

（３）体重

体重の推移を FIGURE 11,12 に示した。

雌雄ともに最高用量の 10000ppm群において、体重増加の抑制が認められた。それらの体重の抑制率は対照群と比べ、最大で雄30%、雌18%であった。

TABLE 12 INCIDENCE OF EXTERNAL AND INTERNAL MASS IN CLINICAL OBSERVATION IN MALE MICE

| Time of mass occurrence (week) | 0~13 | 14~26 | 27~39 | 40~52 | 53~65 | 66~78 | 79~91 | 92~104 | 0~104 |
|---|---|---|---|---|---|---|---|---|---|
| External mass (Includeing Mass[Oral cavity]) | | | | | | | | | |
| Control | 0/50 | 0/50 | 0/50 | 0/50 | 2/48 | 1/43 | 2/42 | 4/40 | 5/50 ( 1/15) |
| 400 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 2/50 | 2/49 | 5/48 | 8/47 | 8/50 ( 2/ 8) |
| 2000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 1/49 | 1/48 | 3/48 | 3/43 | 4/50 ( 1/12) |
| 10000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 1/49 | 3/49 | 4/47 | 9/41 | 11/50 ( 8/17) |
| Mass [Oral cavity] | | | | | | | | | |
| Control | 0/50 | 0/50 | 0/50 | 0/50 | 0/48 | 0/43 | 0/42 | 0/40 | 0/50 ( 0/15) |
| 400 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/49 | 0/48 | 0/47 | 0/50 ( 0/ 8) |
| 2000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/49 | 0/48 | 0/48 | 0/43 | 0/50 ( 0/12) |
| 10000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/49 | 1/49 | 3/47 | 5/41 | 6/50 ( 5/17) |
| Internal mass | | | | | | | | | |
| Control | 1/50 | 2/50 | 3/50 | 2/50 | 1/48 | 1/43 | 1/42 | 6/40 | 10/50 ( 6/15) |
| 400 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 7/49 | 6/48 | 12/47 | 18/50 ( 7/ 8) |
| 2000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/49 | 1/48 | 4/48 | 9/43 | 11/50 ( 3/12) |
| 10000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/49 | 3/49 | 4/47 | 2/41 | 5/50 ( 4/17) |

No. of animals with mass/No. of survival animals at first week on each period.
(No. of dead and moribund animals with mass/No. of dead and moribund animals.)

TABLE 13 INCIDENCE OF EXTERNAL AND INTERNAL MASS IN CLINICAL OBSERVATION IN FEMALE MICE

| Time of mass occurrence (week) | 0~13 | 14~26 | 27~39 | 40~52 | 3~65 | 66~78 | 79~91 | 92~104 | 0~104 |
|---|---|---|---|---|---|---|---|---|---|
| External mass (Includeing Mass[Oral cavity]) | | | | | | | | | |
| Control | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 2/49 | 3/43 | 4/34 | 6/50 ( 5/24) |
| 400 ppm | 0/50 | 0/49 | 0/48 | 0/48 | 1/48 | 0/42 | 1/40 | 4/37 | 5/50 ( 4/23) |
| 2000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 1/48 | 1/44 | 1/35 | 3/50 ( 2/25) |
| 10000 ppm | 0/50 | 0/49 | 0/49 | 0/49 | 0/48 | 2/47 | 2/42 | 8/37 | 10/50 ( 6/26) |
| Mass [Oral cavity] | | | | | | | | | |
| Control | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/49 | 0/43 | 0/34 | 0/50 ( 0/24) |
| 400 ppm | 0/50 | 0/49 | 0/48 | 0/48 | 0/48 | 0/43 | 0/40 | 0/37 | 0/50 ( 0/23) |
| 2000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 0/48 | 0/44 | 0/35 | 0/50 ( 0/25) |
| 10000 ppm | 0/50 | 0/49 | 0/49 | 0/49 | 0/48 | 0/47 | 0/42 | 6/37 | 6/50 ( 4/26) |
| Internal mass | | | | | | | | | |
| Control | 0/50 | 0/50 | 0/50 | 0/50 | 1/50 | 1/49 | 5/43 | 5/34 | 8/50 ( 7/24) |
| 400 ppm | 0/50 | 0/49 | 0/48 | 2/48 | 5/48 | 1/43 | 2/40 | 2/37 | 8/50 ( 7/23) |
| 2000 ppm | 0/50 | 0/50 | 0/50 | 0/50 | 0/50 | 2/48 | 8/44 | 7/35 | 11/50 (10/25) |
| 10000 ppm | 0/50 | 0/49 | 0/49 | 1/49 | 3/48 | 4/47 | 2/42 | 6/37 | 12/50 (10/26) |

No. of animals with mass/No. of survival animals at first week on each period.
(No. of dead and moribund animals with mass/No. of dead and moribund animals.)

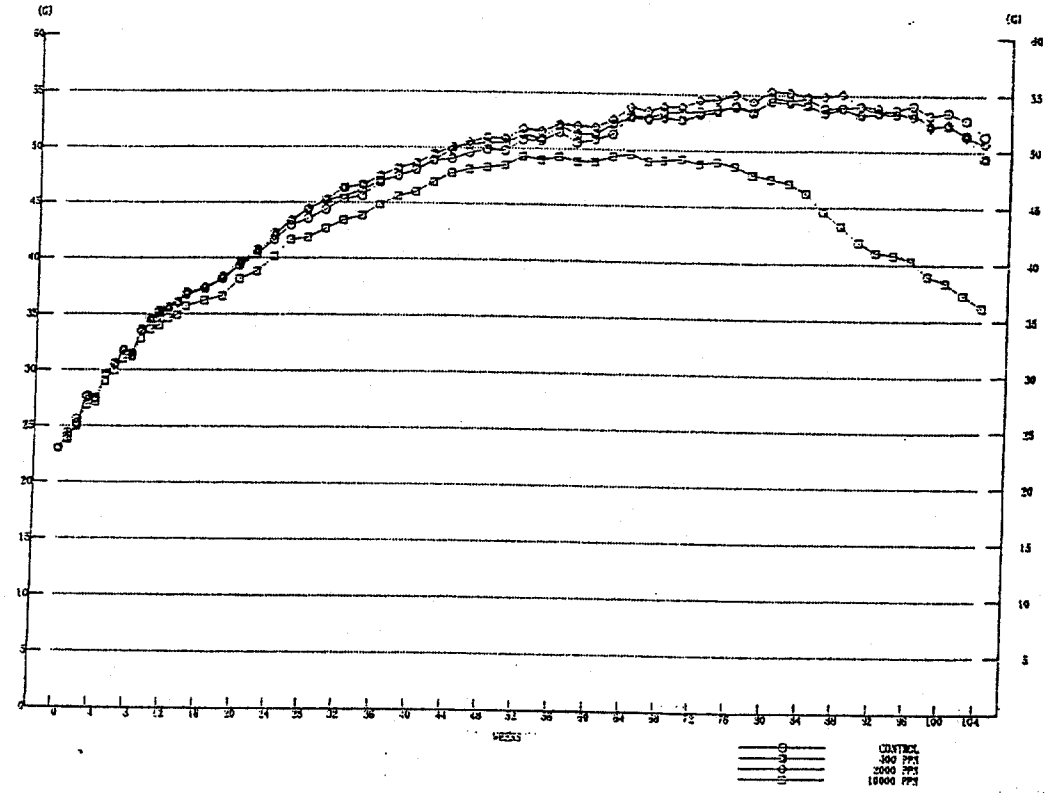

FIGURE 11 BODY WEIGHT CHANGES (MOUSE:MALE)

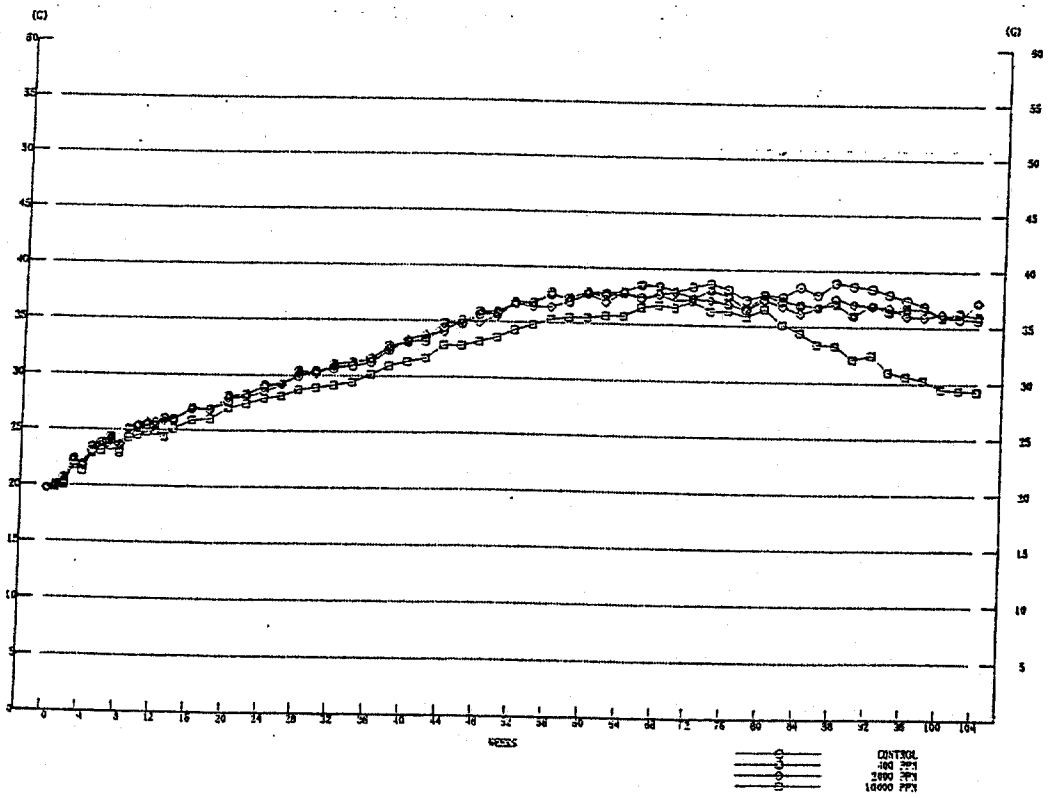

FIGURE 12 BODY WEIGHT CHANGES (MOUSE:FEMALE)

（4）摂水量

摂水量を FIGURE 13,14 に示した。

雄では 10000ppm群にのみ、雌では全投与群で投与量に対応した摂水量の低下が認められた。

それらの群の全投与期間中の平均摂水量は、対照群に対して雄では10000ppm群：90%、雌では400ppm群：96%、2000ppm群：92%、10000ppm群：84%であった。

（5）摂餌量

摂餌量を FIGURE 15,16 に示した。

雌雄ともに投与群の摂餌量は対照群と比較して顕著な差は認められなかった。

（6）被験物質摂取量

体重、摂水量及び設定濃度より被験物質の摂取量を算出した。

被験物質の1日当たりの摂取量は、雄で 400ppm群：0.032～0.085g/kg、2000ppm群：0.167～0.405g/kg、10000ppm群：0.800～2.081g/kg、雌では 400ppm群：0.045～0.125g/kg、2000ppm群：0.230～0.483g/kg、10000ppm群：1.024～2.185g/kgであった。

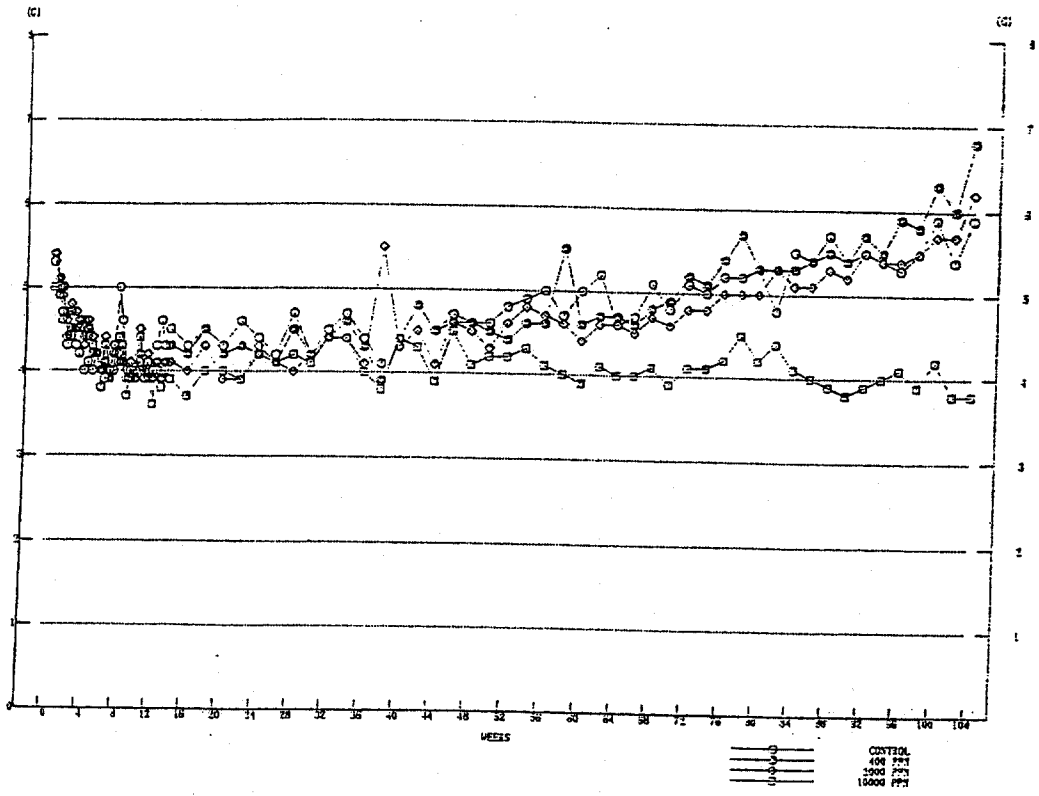

FIGURE 13 WATER CONSUMPTION CHANGES (MOUSE:MALE)

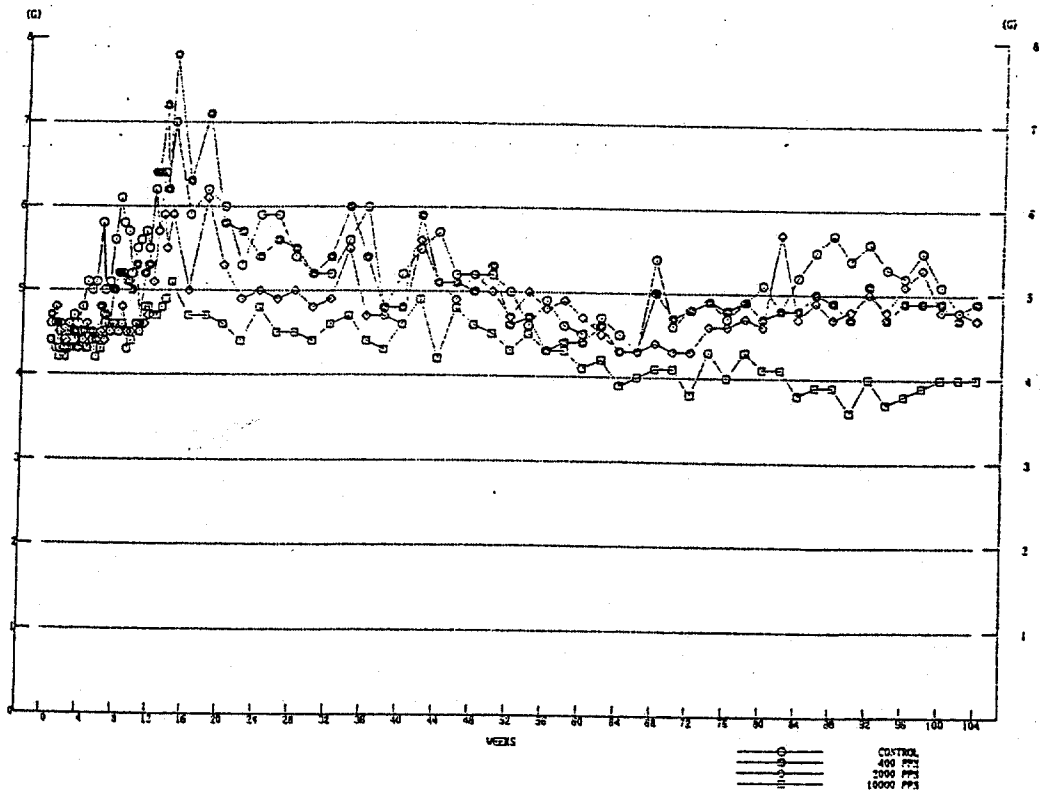

FIGURE 14 WATER CONSUMPTION CHANGES (MOUSE:FEMALE)

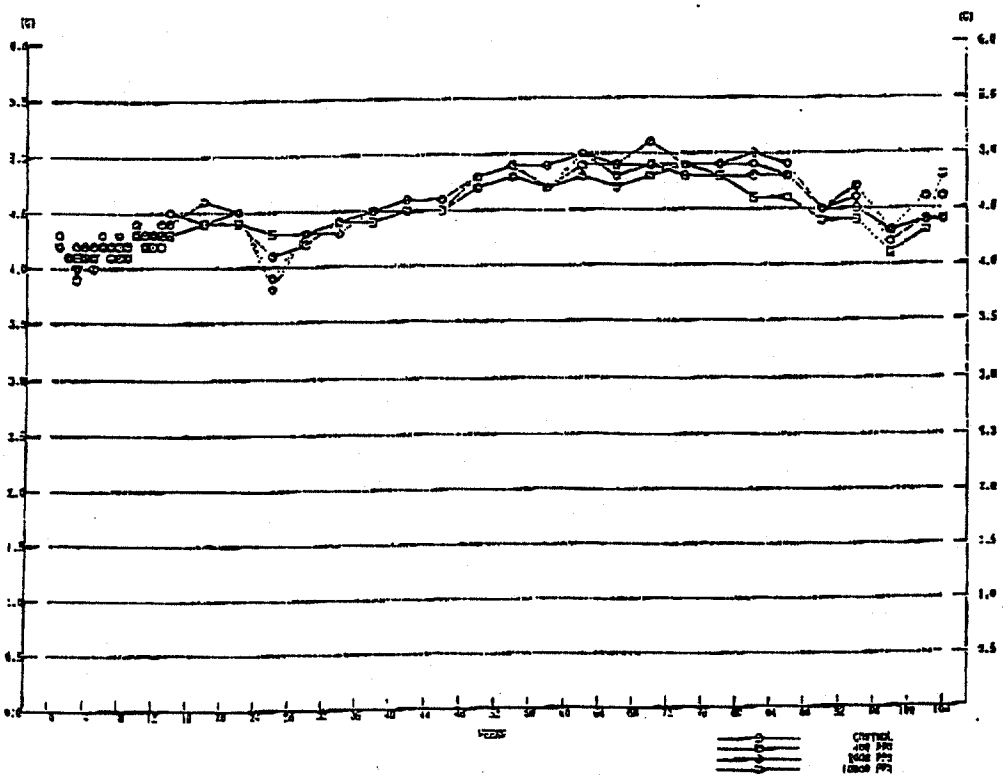

FIGURE 15 FOOD CONSUMPTION CHANGES (MOUSE:MALE)

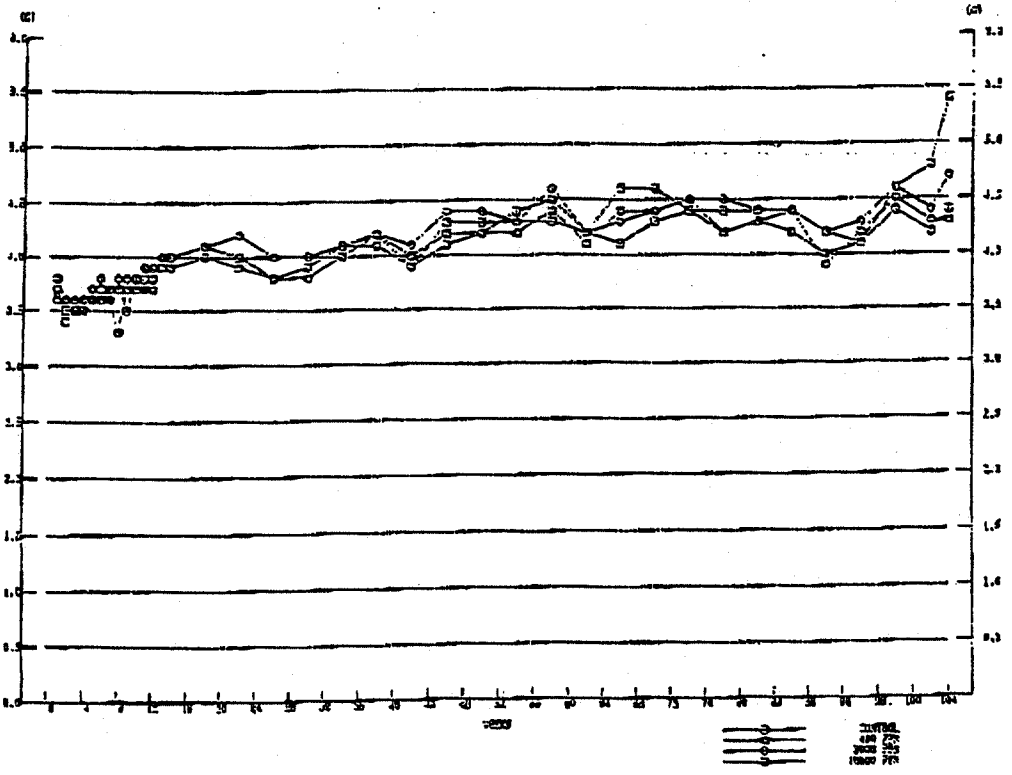

FIGURE 16 FOOD CONSUMPTION CHANGES (MOUSE:FEMALE)

Ⅲ－２－２　血液学的検査・血液生化学的検査・尿検査

（１）血液学的検査

雄では、最高用量の 10000ppm群で血小板数と分葉核好中球比の増加、並びにリンパ球比の減少が認められた。

雌では、最高用量の 10000ppm群でＭＣＨＣの減少が認められた。

（２）血液生化学的検査

雄では、400ppm、10000ppm群でグルコースの減少、10000ppm群でＡ／Ｇ比の増加とＡＬＰ活性の増加、並びに総コレステロール、トリグリセライド及びカルシウムの減少が認められた。

雌では、最高用量の 10000ppm群でグルコースの減少が認められた。

（３）尿検査

雄では、最高用量の 10000ppm群でｐＨの低下と尿蛋白の増加が認められた。

雌では、最高用量の 10000ppm群で尿蛋白の増加とケトン体の増加が認められた。

Ⅲ－２－３　病理学的検査

（１）剖検

解剖時に観察された剖検所見では 10000ppm群に、下顎の結節が雄1/50例と雌5/50例に、また、上顎の結節が雄3/50例と雌1/50例に観察された。

（２）臓器重量

雄では、10000ppm群に解剖時体重の低値に伴って、心臓、肺、腎臓、及び肝臓の実重量の低値、並びに精巣、心臓、肺、腎臓、肝臓及び脳の体重比の高値が認められた。

雌では、10000ppm群に、肺、腎臓及び脳の体重比の高値が認められが、これも解剖時の体重の低値に伴う変化と考えられた。

（３）病理組織学的検査

主な腫瘍性病変とそれに関連した非腫瘍性病変を TABLE 14,15 に示した。

－主な腫瘍性病変－

(口腔)

雄では、扁平上皮癌の発生(対照群:0/50例、400ppm群:0/50例、2000ppm群:0/50例、10000ppm群:13/50例)がPeto検定(死亡率法、有病率法、死亡率法＋有病率法)とCochran-Armitage検定で増加傾向が認められ、Fisher検定でも10000ppm群と対照群の間に有意な増加を示した。また、扁平上皮乳頭腫の発生(対照群:0/50例、400ppm群:0/50例、2000ppm群:0/50例、10000ppm群:4/50例)がPeto検定(有病率法)とCochran-Armitage検定で増加傾向を示した。なお、扁平上皮癌と扁平上皮乳頭腫を合わせた発生(対照群:0/50例、400ppm群:0/50例、2000ppm群:0/50例、10000ppm群:16/50例)も、Peto検定(死亡率法、有病率法、死亡率法＋有病率法)及びCochran-Armitage検定で増加傾向が認められ、Fisher検定でも

Table 14 SELECTED LESIONS OF DIGESTIVE SYSTEM AND LARYNX IN MALE MICE

| | neoplastic disease | | | | | | | | non neoplastic disease | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | squamous cell papilloma | | | | squamous cell carcinoma | | | | squamous cell hyperplasia | | | | basal cell activation | | | | epithlial dysprasia | | | |
| Group(ppm) | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 |
| Number of examined | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| oral cavity | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 12 | 0 | 0 | 2 | 13 | 0 | 0 | 1 | 18 | 0 | 0 | 0 | 24 |
| esophagus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 2 |
| stomach | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 7 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| larynx | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 2 |

Table 15 SELECTED LESIONS OF DIGESTIVE SYSTEM AND LARYNX IN FEMALE MICE

| | neoplastic disease | | | | | | | | non neoplastic disease | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | squamous cell papilloma | | | | squamous cell carcinoma | | | | squamous cell hyperplasia | | | | basal cell activation | | | | epithlial dysprasia | | | |
| Group(ppm) | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 |
| Number of examined | 50 | 50 | 50 | 49 | 50 | 50 | 50 | 49 | 50 | 50 | 50 | 49 | 50 | 50 | 50 | 49 | 50 | 50 | 50 | 49 |
| oral cavity | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 15 | 0 | 0 | 1 | 6 | 0 | 0 | 1 | 17 | 0 | 0 | 0 | 17 |
| esophagus | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 7 |
| stomach | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 3 | 0 | 2 | 0 | 4 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| larynx | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 3 |

10000ppm群で有意な増加を示した。雌では扁平上皮癌の発生(対照群:0/50例、400ppm群:0/50例、2000ppm群:0/50例、10000ppm群:15/49例)が、Peto検定(死亡率法、有病率法、死亡率法＋有病率法)とCochran-Armitage検定で増加傾向を示し、Fisher検定でも 10000ppm群と対照群の間に有意な増加を認めた。また、扁平上皮乳頭腫の発生(対照群:0/50例、400ppm群:0/50例、2000ppm群:0/50例、10000ppm群:3/49例)が、Peto検定(有病率法)とCochran-Armitage検定で増加傾向を示した。なお、扁平上皮癌と扁平上皮乳頭腫を合わせた発生(対照群:0/50例、400ppm群:0/50例、2000ppm群:0/50例、10000ppm群:18/49例)も、Peto検定(死亡率法、有病率法、死亡率法＋有病率法)及びCochran-Armitage検定で増加傾向を示し、Fisher検定でも 10000ppm群で有意な増加を認めた。

また、扁平上皮癌の転移が雌雄の 10000ppm群の肺とリンパ節に各2例と雌の 10000ppm群の唾液腺に1/50例認められた(TABLE 14,15,16,17)。

TABLE 16 NEOPLASTIC LESIONS (ORAL CAVITY) INCIDENCE AND STATISTICAL ANALYSIS IN MALE MICE

| Group Name | Control | 400 ppm | 2000 ppm | 10000 ppm |
|---|---|---|---|---|
| SITE : oral cavity | | | | |
| TUMOUR : squamous cell papilloma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 4/50 (10.0) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 9.76 |
| Terminal Rates(c) | 0/35 ( 0.0) | 0/42 ( 0.0) | 0/38 ( 0.0) | 3/33 ( 9.1) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=------ | | | |
| Prevalence Method(d) | P=0.0003**? | | | |
| Combined analysis(d) | P=------ | | | |
| Cochran-Armitage Test(e) | P=0.0006** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.0688 |
| SITE : oral cavity | | | | |
| TUMOUR : squamous cell carcinnoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 13/50 (26.0) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 17.07 |
| Terminal Rates(c) | 0/35 ( 0.0) | 0/42 ( 0.0) | 0/38 ( 0.0) | 4/33 (12.1) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P<0.0001**? | | | |
| Prevalence Method(d) | P<0.0001**? | | | |
| Combined analysis(d) | P<0.0001**? | | | |
| Cochran-Armitage Test(e) | P<0.0001** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.0003** |
| SITE : oral cavity | | | | |
| TUMOUR : squamous cell papilloma, squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 16/50 (32.0) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 24.39 |
| Terminal Rates(c) | 0/35 ( 0.0) | 0/42 ( 0.0) | 0/33 ( 0.0) | 7/33 (21.2) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P<0.0001**? | | | |
| Prevalence Method(d) | P<0.0001**? | | | |
| Combined analysis(d) | P<0.0001**? | | | |
| Cochran-Armitage Test(e) | P<0.0001** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P<0.0001** |

(a) :Number of tumor-bearing animals/number of animals examined at the site.
(b) :Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c) :Observed tumor incidence at terminal kill.
(d) :Beneth the control incidence are the P-values associated with the trend test.
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combined analysis : Death analysis + Incidental tumor test
(e) :The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates.
? :The conditional probabilities of the largest and smallest possible out comes can not estimated or this P-value isyond the estimated P-value.
------: There is no data which should be statistical analysis.
Significant difference : * : $P \leq 0.05$ ** : $P \leq 0.01$

TABLE 17 NEOPLASTIC LESIONS (ORAL CAVITY) INCIDENCE AND STATISTICAL ANALYSIS IN FEMALE MICE

| Group Name | Control | 400 ppm | 2000 ppm | 10000 ppm |
|---|---|---|---|---|
| SITE : oral cavity | | | | |
| TUMOUR : squamous cell papilloma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 3/49 ( 6.1) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 12.50 |
| Terminal Rates(c) | 0/26 ( 0.0) | 0/27 ( 0.0) | 0/25 ( 0.0) | 2/23 ( 8.7) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=——— | | | |
| Prevalence Method(d) | P=0.0014**? | | | |
| Combined analysis(d) | P=——— | | | |
| Cochran-Armitage Test(e) | P=0.0027** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.1287 |
| SITE : oral cavity | | | | |
| TUMOUR : squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 15/49 (30.6) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 35.48 |
| Terminal Rates(c) | 0/26 ( 0.0) | 0/27 ( 0.0) | 0/25 ( 0.0) | 8/23 (34.8) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.0004**? | | | |
| Prevalence Method(d) | P<0.0001**? | | | |
| Combined analysis(d) | P<0.0001**? | | | |
| Cochran-Armitage Test(e) | P<0.0001** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.0001** |
| SITE : oral cavity | | | | |
| TUMOUR : squamous cell papilloma, squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 18/49 (36.7) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 45.83 |
| Terminal Rates(c) | 0/26 ( 0.0) | 0/27 ( 0.0) | 0/25 ( 0.0) | 7/23 (43.5) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.0004**? | | | |
| Prevalence Method(d) | P<0.0001**? | | | |
| Combined analysis(d) | P<0.0001**? | | | |
| Cochran-Armitage Test(e) | P<0.0001** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P<0.0001** |

(a):Number of tumor-bearing animals/number of animals examined at the site.
(b):Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c):Observed tumor incidence at terminal kill.
(d):Beneth the control incidence are the P-values associated with the trend test.
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combined analysis : Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates.
? :The conditional probabilities of the largest and smallest possible out comes can not estimated or this P-value isyond the estimated P-value.
———: There is no data which should be statistical analysis.
Significant difference : * : $P \leq 0.05$ ** : $P \leq 0.01$

（食道）

雄では扁平上皮癌の発生(対照群:0/50例、400ppm群:0/50例、2000ppm群:0/50例、10000ppm群:7/50例)が、Peto検定(有病率法、死亡率法＋有病率法)とCochran-Armitage検定で増加傾向を示し、Fisher検定でも 10000ppm群で有意な増加を認めた。雌では扁平上皮乳頭腫が 2000ppm群の1/50例に、扁平上皮癌が 10000ppm群の1/50例に認められた。なお、扁平上皮癌の転移が雄の 10000ppm群の肺に1/50例認められた(TABLE 14,15,18)。

TABLE 18　NEOPLASTIC LESIONS (ESOPHAGUS) INCIDENCE AND STATISTICAL ANALYSIS IN MALE MICE

| Group Name | Control | 400 ppm | 2000 ppm | 10000 ppm |
|---|---|---|---|---|
| SITE : esophagus | | | | |
| TUMOUR : squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 7/50 (14.0) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 15.15 |
| Terminal Rates(c) | 0/35 ( 0.0) | 0/42 ( 0.0) | 0/38 ( 0.0) | 5/33 (15.2) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.1801 | | | |
| Prevalence Method(d) | P<0.0001**? | | | |
| Combined analysis(d) | P<0.0001**? | | | |
| Cochran-Armitage Test(e) | P<0.0001** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.0101* |

(a):Number of tumor-bearing animals/number of animals examined at the site.
(b):Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c):Observed tumor incidence at terminal kill.
(d):Beneth the control incidence are the P-values associated with the trend test.
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combined analysis : Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates.
? :The conditional probabilities of the largest and smallest possible out comes can not estimated or this P-value beyond is the estimated P-value.
Significant difference ; * : P ≦ 0.05　** : P ≦ 0.01

（胃）

雄では扁平上皮癌の発生(対照群:1/50例、400ppm群:0/50例、2000ppm群:0/50例、10000ppm群:7/50例)が、Peto検定(有病率法、死亡率法＋有病率法)とCochran-Armitage検定で増加傾向を示し、Fisher検定でも 10000ppm群で有意な増加を認めた。また、扁平上皮乳頭腫は 10000ppm群の2/50例に認められた。なお、扁平上皮癌と扁平上皮乳頭腫を合わせた発生(対照群:1/50例、400ppm群:0/50例、2000ppm群:0/50例、10000ppm群:9/50例)も、Peto検定(有病率法、死亡率法＋有病率法)とCochran-Armitage検定で増加傾向を示し、Fisher検定でも 10000ppm群で有意な増加を認めた。

雌では扁平上皮癌の発生(対照群:0/50例、400ppm群:0/50例、2000ppm群:0/50例、10000ppm群:3/49例)が、Peto検定(死亡率法、死亡率法＋有病率法)とCochran-Armitage検定で増加傾向を示した。また、扁平上皮乳頭腫は10000ppm群に1/50例認められた。なお、扁平

上皮癌と扁平上皮乳頭腫を合わせた発生(対照群:0/50例、400ppm群:0/50例、2000ppm群:0/50例、10000ppm群:4/49例)も、Peto検定(死亡率法、有病率法、死亡率法+有病率法)とCochran-Armitage検定で増加傾向を認めた。なお、いずれの腫瘍も前胃から発生していた。また、扁平上皮癌の転移が雌雄の 10000ppm群の肝臓に各1例、ならびに 10000ppm群の雌の腎臓、膵臓及びリンパ節に各1例認められた(TABLE 14,15,19,20)。

TABLE 19 NEOPLASTIC LESIONS (STOMACH) INCIDENCE AND STATISTICAL ANALYSIS MALE MICE

| Group Name | Control | 400 ppm | 2000 ppm | 10000 ppm |
|---|---|---|---|---|
| SITE : stomach | | | | |
| TUMOUR : squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 1/50 ( 2.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 7/50 (14.0) |
| Adjusted Rates(b) | 2.86 | 0.0 | 0.0 | 18.18 |
| Terminal Rates(c) | 1/35 ( 2.9) | 0/42 ( 0.0) | 0/38 ( 0.0) | 6/33 (18.2) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.1821 | | | |
| Prevalence Method(d) | P=0.0001** | | | |
| Combined analysis(d) | P<0.0001** | | | |
| Cochran-Armitage Test(e) | P=0.0001** | | | |
| Fisher Exact Test(e) | | P=0.4950 | P=0.4950 | P=0.0430* |
| SITE : stomach | | | | |
| TUMOUR : squamous cell papilloma, squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 1/50 ( 2.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 9/50 (18.0) |
| Adjusted Rates(b) | 2.86 | 0.0 | 0.0 | 24.24 |
| Terminal Rates(c) | 1/35 ( 2.9) | 0/42 ( 0.0) | 0/38 ( 0.0) | 8/33 (24.2) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.1821 | | | |
| Prevalence Method(d) | P=0.0001** | | | |
| Combined analysis(d) | P<0.0001** | | | |
| Cochran-Armitage Test(e) | P=0.0001** | | | |
| Fisher Exact Test(e) | | P=0.4950 | P=0.4950 | P=0.0150* |

(a):Number of tumor-bearing animals/number of animals examined at the site.
(b):Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c):Observed tumor incidence at terminal kill.
(d):Beneth the control incidence are the P-values associated with the trend test.
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combined analysis : Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates.
Significant difference ; * : $P \leq 0.05$ ** : $P \leq 0.01$

TABLE 20 NEOPLASTIC LESIONS (STOMACH) INCIDENCE AND STATISTICAL ANALYSIS IN FEMALE MICE

| Group Name | Control | 400 ppm | 2000 ppm | 10000 ppm |
|---|---|---|---|---|
| SITE : stomach | | | | |
| TUMOUR : squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 3/49 ( 6.1) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 4.35 |
| Terminal Rates(c) | 0/26 ( 0.0) | 0/27 ( 0.0) | 0/25 ( 0.0) | 1/23 ( 4.3) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.0146* ? | | | |
| Prevalence Method(d) | P=0.1561 | | | |
| Combined analysis(d) | P=0.0019**? | | | |
| Cochran-Armitage Test(e) | P=0.0027** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.1287 |
| SITE : stomach | | | | |
| TUMOUR : squamous cell papilloma, squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 4/49 ( 8.2) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 8.70 |
| Terminal Rates(c) | 0/26 ( 0.0) | 0/27 ( 0.0) | 0/25 ( 0.0) | 2/23 ( 8.7) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.0146* ? | | | |
| Prevalence Method(d) | P=0.0103* ? | | | |
| Combined analysis(d) | P=0.0002**? | | | |
| Cochran-Armitage Test(e) | P=0.0005** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.0662** |

(a):Number of tumor-bearing animals/number of animals examined at the site.
(b):Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c):Observed tumor incidence at terminal kill.
(d):Beneth the control incidence are the P-values associated with the trend test.
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combined analysis : Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates.
? :The conditional probabilities of the largest and smallest possible out comes can not estimated or this P-value beyond is the estimated P-value.
Significant difference ; * : $P \leq 0.05$ ** : $P \leq 0.01$

(喉頭)

扁平上皮癌が 2000ppm群の雌に1/50例、及び 10000ppm群の雄に2/50例と雌に1/50例に認められた。なお、2000ppm群の雌1例は食道の扁平上皮乳頭腫を観察した例と同一個体であった(TABLE 14,15)。

(肝臓)

雄の肝細胞癌の発生(対照群:13/50例、400ppm群:10/50例、2000ppm群:9/50例、10000ppm群:4/50例)がCochran-Armitage検定で減少傾向を示し、Fisher検定でも10000ppm群で有意な減少を認めた(TABLE 21)。

TABLE 21 NEOPLASTIC LESIONS (LIVER) INCIDENCE AND STATISTICAL ANALYSISIN MALE MICE

| Group Name | Control | 400 ppm | 2000 ppm | 10000 ppm |
|---|---|---|---|---|
| SITE : liver | | | | |
| TUMOUR : hepatocellular carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 13/50 (26.0) | 10/50 (20.0) | 9/50 (18.0) | 4/50 ( 8.0) |
| Adjusted Rates(b) | 31.43 | 23.26 | 23.68 | 11.76 |
| Terminal Rates(c) | 11/35 (31.4) | 9/42 (21.4) | 9/38 (23.7) | 3/33 ( 9.1) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=1.0000 ? | | | |
| Prevalence Method(d) | P=0.9783 | | | |
| Combined analysis(d) | P=0.9837 | | | |
| Cochran-Armitage Test(e) | P=0.0243* | | | |
| Fisher Exact Test(e) | | P=0.3703 | P=0.2965 | P=0.0371* |

(a) :Number of tumor-bearing animals/number of animals examined at the site.
(b) :Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c) :Observed tumor incidence at terminal kill.
(d) :Beneth the control incidence are the P-values associated with the trend test.
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combined analysis : Death analysis + Incidental tumor test
(e) :The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates.
? :The conditional probabilities of the largest and smallest possible out comes can not estimated or this P-value beyond the estimated P-value.
Significant difference : * : $P \leq 0.05$ ** : $P \leq 0.01$

－非腫瘍性病変－

(口腔)

扁平上皮過形成が 2000ppm群の雄2/50例と雌1/50例及び 10000ppm群の雄 13/50例と雌6/50例に認められた。また、基底細胞の賦活化が 2000ppm群の雌雄各1/50例、及び 10000ppm群の雄18/50例と雌17/49例に認められた。さらに、上皮の異形成が、10000ppm群の雄24/50例と雌17/49例に認められた。なお、いずれの所見も雌雄ともに定期解剖例で、10000ppm群と対照群の間に統計的に有意な差を示した(TABLE 14,15)。

(食道)

基底細胞の賦活化が、10000ppm群の雄9/50例と雌15/49例に認められた。また、扁平上皮過形成が 10000ppm群の雄2例と雌2/49例に認められた。さらに、上皮の異形成が雄2/50例と雌7/49例に認められた。なお、基底細胞の賦活化の発生は雌雄の定期解剖例と雌の途中死亡／瀕死例で、10000ppm群と対照群の間に統計的に有意な増加を示した(TABLE 14,15)。

（胃）

基底細胞の賦活化が、10000ppm群の雄1/50例と雌1/49例に認められ、上皮異形成が10000ppm群の雄1/50例に認められた(TABLE 14,15)。

また、雄の定期解剖例で腺胃の過形成の発生(対照群:25/35例、400ppm群:31/42例、2000ppm群:26/38例、10000ppm群:11/33例)が 10000ppm群で対照群に比較して統計的に有意な減少を示した。

なお、前胃の過形成が雌雄の 10000ppm群と雌の 400ppm群に認められた。しかし、この所見は一般に加齢性の変化としても認められることより、酢酸ビニルの投与による影響と断定できなかった。

（喉頭）

基底細胞の賦活化が、10000ppm群の雄3/50例と雌6/49例に認められた。また、上皮の異形成が 10000ppm群の雄2/50例と雌3/49例に認められ、扁平上皮過形成が雄1/50例に認められた。なお、基底細胞の賦活化の発生は雌の定期解剖例で 10000ppm群と対照群の間に統計的に有意な増加を示した(TABLE 14,15)。

（唾液腺）

唾液腺の萎縮が 10000ppm群の雄6/50例と雌4/49例に認められた。

（鼻腔）

雄の鼻腺の呼吸上皮化生の発生(対照群:26/35例、400ppm群:28/42例、2000ppm群:26/38例、10000ppm群:12/33例)が、定期解剖例で 10000ppm群に統計的に有意な発生低下を示した。

（脳）

雄の定期解剖例で鉱質沈着の発生(対照群:8/50例、400ppm群:17/50例、2000ppm群:16/50例、10000ppm群:19/50例)が、定期解剖例で 10000ppm群に統計的に有意な発生増加を示した。

－その他の非腫瘍性病変－

その他の非腫瘍性病変としては定期解剖例の雄で腎臓の好塩基性変化の低下、近位尿細管上皮の空胞化の増加及び精巣の鉱質沈着の増加、雌では鼻腔の呼吸上皮のエオジン好性変化の増加と子宮の嚢胞状内膜増生の増加が認められた。しかし、これらの所見の発生率は投与濃度に対応したものではなく、被験物質の投与による影響とは考えられなかった。

（4）死因

病理学的にみた死亡／瀕死の原因を TABLE 22 に示した。

口腔の腫瘍による死亡が 10000ppm群の雄6例、雌4例に認められた。また、胃の腫瘍及び喉頭の腫瘍による死亡が雌雄ともに 10000ppm群に少数例認められ、食道の腫瘍による死亡が 10000ppm群の雄1例に認められた。

TABLE 22 CAUSE OF DEATH IN MICE

| | Male | | | | Female | | | |
|---|---|---|---|---|---|---|---|---|
| Group | Control | 400ppm | 2000ppm | 10000ppm | Control | 400ppm | 2000ppm | 10000ppm |
| Number of dead/moribund animal | 15 | 8 | 12 | 17 | 24 | 23 | 25 | 26 |
| No microscopical confirmation | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 |
| Cerdiovascular lesion | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Urinary system lesion | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Circulatory disorder | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| Urinary retention | 3 | 1 | 0 | 1 | 0 | 0 | 1 | 0 |
| Arteritis | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hydronephrosis | 2 | 0 | 0 | 1 | 1 | 0 | 1 | 1 |
| Tumor death : leukemia | 1 | 3 | 3 | 1 | 7 | 9 | 11 | 9 |
| : subcutis | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| : larynx | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| : lung | 1 | 0 | 2 | 1 | 0 | 1 | 0 | 0 |
| : spleen | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| : oral cavity | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 4 |
| : toung | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| : salivary gland | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| : esophagus | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| : stomach | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| : liver | 1 | 2 | 2 | 3 | 0 | 1 | 1 | 0 |
| : urinary bladder | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| : pituitary gland | 2 | 0 | 0 | 0 | 2 | 0 | 1 | 1 |
| : epididymis | 1 | 1 | 0 | 1 | - | - | - | - |
| : seminal vesicle | 0 | 0 | 1 | 0 | - | - | - | - |
| : ovary | - | - | - | - | 0 | 0 | 0 | 1 |
| : uterus | - | - | - | - | 9 | 8 | 7 | 4 |
| : mammary gland | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| : peripheral nerve | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| : muscle | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 |
| : bone | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| : retroperiteneum | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

## Ⅳ　考察

### Ⅳ－１ラット

－生死状況等－

投与群の生存率は雌雄とも対照群とくらべて顕著な差は認められなかった。雌雄とも最高用量の 10000ppm群で体重増加のわずかな抑制、摂水量の低下が認められた。さらに、雌雄とも 10000ppm群では血液生化学的検査で総コレステロールとリン脂質の減少、並びに尿検査でケトン体の疑陽性例の増加等の軽度の栄養障害性の所見が認められた。

－腫瘍性病変－

（１）口腔

雄では、10000ppm群で扁平上皮癌の発生が5/50例に、また扁平上皮乳頭腫が2/50例に認められた。当センターでのF344ラットのヒストリカルコントロールデータ(文献 6)では、口腔の扁平上皮癌については未だに発生を見ておらず、また、扁平上皮乳頭腫については1/550例(0.2%)と、発生は稀であると思われる。よって投与群でのこれらの腫瘍の発生は酢酸ビニルの投与による影響と考えた。なお、扁平上皮癌を示した個体のうち1例は食道に浸潤性の転移を認め、悪性度の強い進行した癌であった。

雌では扁平上皮癌の発生が 400ppmの1/50例、2000ppmの1/50例及び 10000ppm群の3/50例に認められた。口腔の扁平上皮癌の発生は当センターのヒストリカルコントロールデータ(文献 6)やNTPのデータ(文献 7)でも発生が報告されておらず、極めて稀な腫瘍であり、酢酸ビニルの投与による影響と推察された。

（２）食道

扁平上皮癌が 10000ppm群の雌に1/50例に認められた。この腫瘍は当センターのヒストリカルコントロールデータ(文献 6)やNTPのデータ(文献 7)では発生が報告されておらず、極めて稀な腫瘍である。従って、この腫瘍も酢酸ビニルの投与により発生したものと考えられた。

－非腫瘍性病変－

前胃の基底細胞の賦活化が 10000ppm群の定期解剖例で雌5/50例に認められ、対照群と比較して統計的にも有意な増加を示し、酢酸ビニルの投与による影響と考えられた。また、同群においては口腔に基底細胞の賦活化(雄2例と雌1例)と上皮異形成(雌2例)、食道に基底細胞の賦活化（雌4例）と扁平上皮過形成（雌雄各1例）、前胃に基底細胞の賦活化（雄2例）が観察された。これらの変化は、統計的に有意な差を示さなかったものの、加齢性の変化として見られる変化ではなく、また口腔と食道に発生した腫瘍と同様に消化器系器

官の扁平上皮に限定されており、酢酸ビニルの投与による影響と考えられた。

また、腎臓の慢性腎症の程度の低下が雄の 10000ppm群で認められた。加齢性変化としてF344ラットに多発する慢性腎症は、制限給餌によって発生が抑制されることが報告(文献 8)されているが、今回、大幅な摂餌量の抑制や体重増加の抑制はみられなかったので、この原因は制限給餌によるものとは断定できなかった。

なお、口腔や食道と同様に消化器の扁平上皮過形成である前胃の過形成が、2000ppm群の雄1例に観察された。しかし、前胃の過形成は、一般に加齢性の変化としてもみられ、また、今回の結果では対照群にも2例認められることから投与による影響ではないと考えた。

Ⅳ－２マウス

－生死状況等－

投与群の生存率は、雌雄とも対照群と比べ顕著な差は認められなかった。

雌雄とも 10000ppm群において口腔の腫瘍を死因とする例が多くみられた。また、少数例ではあるが胃の腫瘍と喉頭の腫瘍による死亡が雌雄の 10000ppm群に、食道の腫瘍による死亡が雄の 10000ppm群にみられた。

体重増加の抑制が雌雄とも 10000ppm群で、摂水量の低下が雄では 10000ppm群、雌では全投与群に認められた。また、血液生化学的検査で、雄では、10000ppm群にグルコース、総コレステロール及びトリグリセライドの減少、雌でも 10000ppm群にグルコースの減少が認められた。さらに、尿検査では、雄の 10000ppm群にｐＨの低下と尿蛋白の陽性度の増加、雌の 10000ppm群に尿蛋白の陽性度の増加とケトン体の陽性例の増加が認められ、栄養障害が疑われた。

－腫瘍性病変－

（１）口腔

雄では扁平上皮癌の発生が 10000ppm群の13/50例に、扁平上皮乳頭腫の発生が 10000ppm群の4/50例に認められた。雌でも扁平上皮癌の発生が 10000ppm群の15/49例に、扁平上皮乳頭腫の発生が 10000ppm群の3/49例に認められた。これらの腫瘍は当センターにおけるヒストリカルコントロールデータ（文献 6)では発生が認められず、極めて稀な腫瘍である。したがってこれらの腫瘍の発生は雌雄とも、酢酸ビニルの投与による影響と考えた。なお、扁平上皮癌の転移が肺、唾液腺、及び近接したリンパ節に認められ、中には一個体で複数の臓器に転移を示すものも認められた。

（2）食道

扁平上皮癌の発生が雄の 10000ppm群で7/50例に認められ、酢酸ビニルの投与の影響と考えられた。また、雄の１例に扁平上皮癌の転移が認められた。

雌では扁平上皮乳頭腫が 2000ppm群の1/50例に、扁平上皮癌が 10000ppm群の1/50例に認められた。これらの腫瘍は当センターにおけるヒストリカルコントロールデータ（文献6)では発生が認められず、自然発生が極めて稀な腫瘍(文献 6,9,10)であることから酢酸ビニルの投与により発生したものと考えられた。

（3）胃

雄の前胃では扁平上皮癌の発生が対照群の1/50例と 10000ppm群の7/50例に、また、扁平上皮乳頭腫が 10000ppm群の2/50例、また雌では扁平上皮癌の発生が 10000ppm群の3/49例に、扁平上皮乳頭腫が 10000ppm群の1/50例に認められた。これらの腫瘍は自然発生が極めて稀な腫瘍（文献 6,9,10）であることから、雌雄とも酢酸ビニルの投与の影響と考えられた。また、発生した扁平上皮癌は浸潤性に増殖するものが多く、また、雌雄各1例に他臓器への転移が認められた。特に雌の1例では腹腔内に広く転移し、肝臓、腎臓、膵臓及び近接したリンパ節に転移巣が認められ悪性度の高い腫瘍と考えられた。

（4）喉頭

扁平上皮癌が 10000ppm群の雄2/50例と雌1/49例に認められ、また、扁平上皮乳頭腫が2000ppm群の雌1/50例に認められた。これらの腫瘍の発生は、少数例ではあるが、自然発生が極めて稀な腫瘍であることから(文献 6,9,10)これらの腫瘍も酢酸ビニルの投与により発生したものと推察される。

（5）肝臓

雄の肝細胞癌の発生(対照群:13/50例、400ppm群:10/50例、2000ppm群:9/50例、10000ppm群:4/50例)が、Cochran-Armitage検定で減少傾向を示し、Fisher検定でも 10000ppm群と対照群の間に有意な減少を示した。

マウスの雄の肝腫瘍は、体重増加の抑制によって発生が抑制されると報告(文献 11)されている。今回の試験でも酢酸ビニルの投与による摂水忌避に伴って、投与群では摂餌量と体重増加が抑制されていることから、肝細胞癌の発生減少は摂水忌避に伴う体重増加の抑制によるものと推察された。

なお、雌については投与群と対照群の発生数に著変は認められなかった。

－非腫瘍性病変－

酢酸ビニルの投与によると考えられる影響として、10000ppm群では口腔、食道及び喉頭

に扁平上皮過形成、基底細胞の賦活化および上皮の異形成、また前胃に基底細胞の賦活化と上皮の異形成が認められた。これらの発生数は臓器により差があり、口腔が最も多かった。さらに、2000ppm群でも、口腔の扁平上皮過形成と基底細胞の賦活化の発生が少数例にみられた。　その他、10000ppm群の雌雄に唾液腺の萎縮が認められたが、口腔の下顎側に腫瘍を持つ個体と一致しており、下顎の腫瘍の発生に伴った二次的な変化と考えた。

上記の所見の他に、対照群と投与群の間に投与量に対応した発生の差が認められ、酢酸ビニルの投与による影響を否定できない所見を以下に示す。

脳の鉱質沈着の発生増加(10000ppm群の雄の定期解剖例)

鼻腺の呼吸上皮化生の発生低下(10000ppm群の雄の定期解剖例)

腺胃の過形成の発生低下(10000ppm群の雄の定期解剖例)

Ⅳ－3　他試験との比較及びまとめ

Bogdanffyらは、酢酸ビニルの吸入投与によるがん原性試験で鼻腔に扁平上皮癌が発生したと報告している(文献 12)。今回の飲水試験においても酢酸ビニルと直接接触する消化器系の臓器、すなわち口腔、食道及び胃、並びに口腔に近接した喉頭に、扁平上皮に由来する腫瘍の発生をみた。このように両試験とも酢酸ビニルは直接的に接触する局所に腫瘍を発生させることを示している。Lijinskyらは酢酸ビニルの 100週間の飲水試験で、肝臓、子宮及び甲状腺のC細胞腺腫の発生を報告している(文献 15)が今回の飲水試験においては甲状腺、肝臓及び子宮とも酢酸ビニルの投与に起因した腫瘍の増加は見られなかった。これらの結果の相違は試験形態、投与濃度、動物数の相違から起こった可能性があるが、Lijinskyらの報告に試験条件等の詳細な記述がないため十分な検討は出来なかった。

非腫瘍性病変では飲水時に酢酸ビニルに直接接触する臓器に、扁平上皮の過形成、基底細胞の賦活化及び上皮の異形成が観察された。

これらの所見は、

①腫瘍の発生増加が認められた臓器に一致している。

②発生部位が扁平上皮であり発生増加が見られた腫瘍の発生母地と同じ組織である。

③細胞の増殖性変化である扁平上皮過形成と基底細胞の賦活化が化学物質の投与により発生した場合、その病変は細胞増殖刺激の持続により、扁平上皮由来の腫瘍を発生させる可能性があるという報告(文献 13)や上皮の異形成は細胞の悪性化に深く関与している前癌病変であるという報告(文献 14)があり、前癌所見として合理的な所見であることから扁平上皮の過形成、基底細胞の賦活化及び上皮の異形性は酢酸ビニルの投与によって発生した扁平上皮癌や扁平上皮乳頭腫の前段階の変化であると考えられる。

## V 結論

F344/DuCrj(Fischer)ラット及びCrj:BDF 1 マウスを用いて酢酸ビニルの2年間（104週間）にわたる飲水の経口投与によるがん原性試験を行った。

ラットでは、雄に口腔の扁平上皮癌と扁平上皮乳頭腫、雌に口腔と食道の扁平上皮癌の発生増加が認められ、腫瘍の発生する濃度は、口腔が雄で10000 ppm、雌で 400ppm以上、食道が雌で 10000ppmであった。

マウスでは、雄に口腔と胃の扁平上皮癌や扁平上皮乳頭腫及び食道と喉頭の扁平上皮癌、また、雌に口腔と胃の扁平上皮癌、扁平上皮乳頭腫及び食道と喉頭の扁平上皮癌の発生増加が認められ、腫瘍の発生する濃度は口腔と胃が雌雄とも 10000ppm、食道と喉頭が雄で 10000ppm、雌で 2000ppmであった。

以上の結果により、酢酸ビニルのF344/DuCrj(Fischer)ラット及びCrj:BDF 1 マウスに対する明らかながん原性が認められた。

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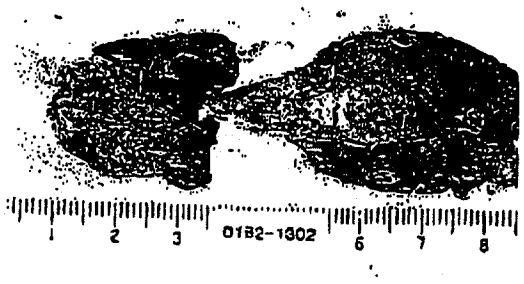


写真　1
下顎、結節（口腔の扁平上皮癌）（↑）
ラット、雄、10000ppm群、動物NO. 0162-1302

写真　2　口腔
扁平上皮癌（↑）
ラット、雌、10000ppm群、動物NO. 0162-2304
(H&E染色、X32)

写真　3　口腔
扁平上皮乳頭腫（↑）
ラット、雄、10000ppm群、動物NO. 0162-2320
(H&E染色、X80)

写真　4　食道
扁平上皮癌（↑）
ラット、雌、10000ppm群、動物NO. 0162-2304
(H&E染色、X32)

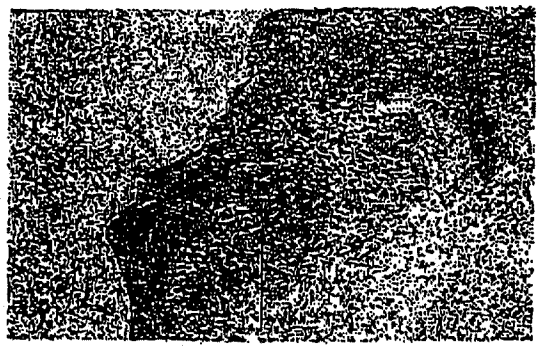

写真　5　口腔
上皮異形成（↑）
ラット、雌、10000ppm群、動物NO. 0162-2304
(H&E染色、X32)

写真　6　胃（前胃）
基底細胞賦活化（↑）
ラット、雄、10000ppm群、動物NO. 0162-1314
(H&E染色、X80)


写真　7
下顎、結節（口腔の扁平上皮癌）（↑）
マウス、雌、10000ppm群、動物NO. 0163-2305

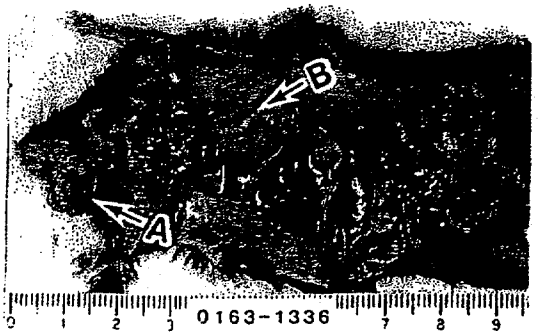


写真　8
下顎、結節（口腔の扁平上皮癌）（A）
肺、結節（転移：口腔腫瘍）（B）
マウス、雄、10000ppm群、動物NO. 0163-1336

写真　9　口腔
扁平上皮癌（↑）
マウス、雄、10000ppm群、動物NO. 0163-1342
(H&E染色、X16)

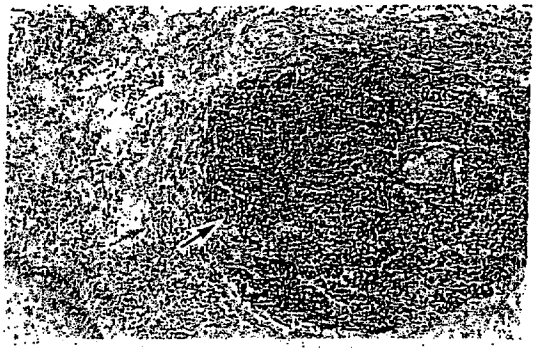

写真 10　肺
転移：口腔腫瘍（↑）
マウス、雄、10000ppm群、動物NO. 0163-1336
(H&E染色、X80)

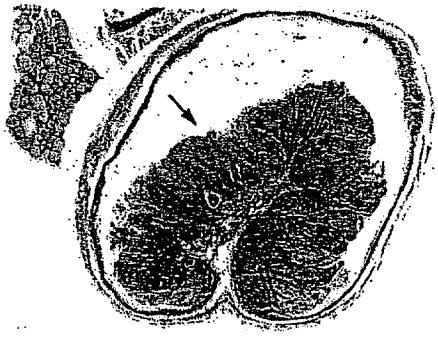

写真 11　食道
扁平上皮癌（外方性増殖）（↑）
マウス、雄、10000ppm群、動物NO. 0163-1317
(H&E染色、X16)

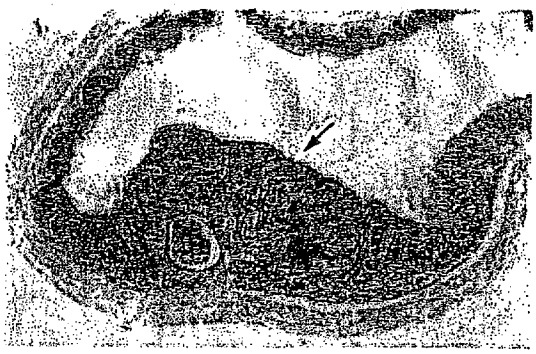

写真 12　食道
扁平上皮癌（内方性増殖）（↑）
マウス、雄、10000ppm群、動物NO. 0163-1349
(H&E染色、X32)

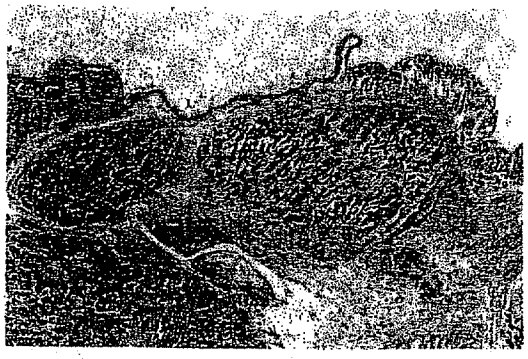

写真 13　胃（前胃）
扁平上皮癌
マウス、雌、10000ppm群、動物NO. 0163-2334
(H&E染色、X16)

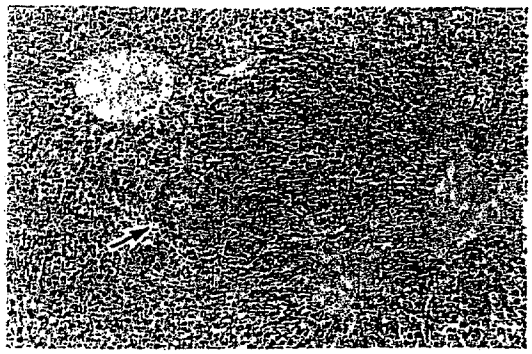

写真 14　肝臓
転移：胃腫瘍（↑）
マウス、雌、10000ppm群、動物NO. 0163-2332
(H&E染色、X80)

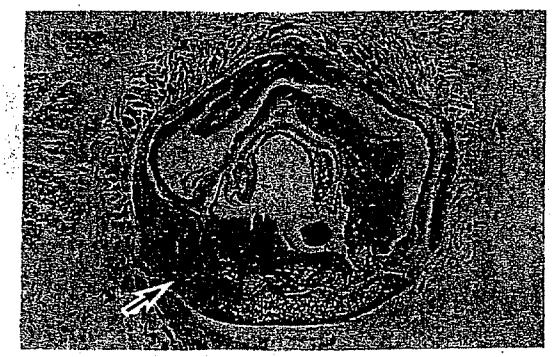

写真 15　喉頭
扁平上皮癌（↑）
マウス、雄、10000ppm群、動物NO. 0163-1326
(H&E染色、X16)

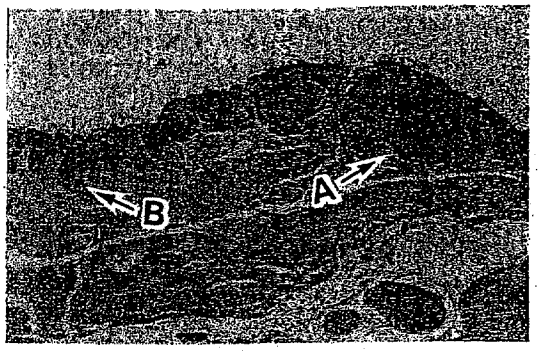


写真 16　口腔
扁平上皮過形成（A）、基底細胞賦活化（B）
マウス、雄、10000ppm群、動物NO. 0163-1342
(H&E染色、X32)

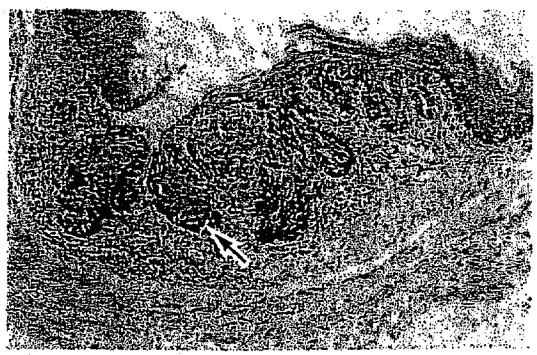

写真 17　食道
上皮異形成（↑）
マウス、雌、10000ppm群、動物NO. 0163-2331
(H&E染色、X80)

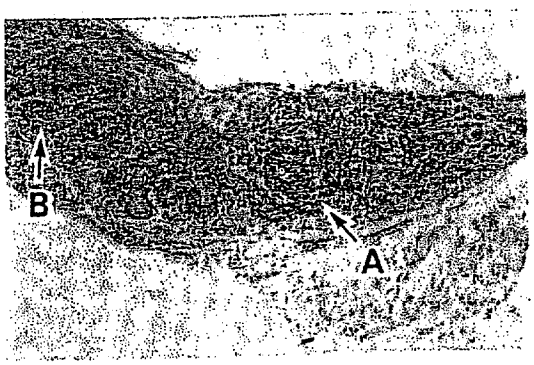


写真 18　喉頭
上皮異形成（A）、基底細胞賦活化（B）
マウス、雌、10000ppm群、動物NO. 0163-2314
(H&E染色、X80)

# Toxicology and Carcinogenesis Studies of Vinyl Acetate in F344/DuCrj Rats and Crj:BDF1 Mice ( Drinking Water Study)

## 1. Study Methods (Table 1)

Groups of 50 male and female of F344/DuCrj rats (Fischer, Charles River Japan, Inc.), six weeks old, received drinking water containing 0, 400, 2000 or 10000 ppm vinyl acetate (98.0%, Wako Pure Chemical co., Tokyo) for 104 weeks. And groups of 50 male and female of Crj:BDF1 mice (Charles River Japan, Inc.), six weeks old, received drinking water containing 0, 400, 2000 or 10000 ppm vinyl acetate for 104 weeks.

## 2. Results

### 1) Rat study

Survival (Fig. 1, 2): There were no significant difference in survival rates at the end of the study between treated groups and control group in both sexes (male; control: 44/50, 400 ppm: 40/50, 2000 ppm: 36/50, 10000 ppm: 39/50, female; control: 41/50, 400 ppm: 40/50, 2000 ppm: 41/50, 10000 ppm: 37/50).

Clinical observation: A few rats of the treated groups had nodules in the oral cavity (male; 2000 ppm: 1/50, 10000 ppm: 2/50, female; 400 ppm: 1/50, 10000 ppm: 1/50). There were no other abnormal clinical signs in the treated males and females.

Body weight (Fig. 3, 4): At the end of the study, body weight of males and females in the 10000ppm group were slightly, but statistically significantly, less than controls (8% and 10%, respectively).

Food and water consumption (Fig. 5 – 8): Food consumption did not show significant variation among groups for both males and females. Water consumption was decreased throughout the experimental period in the 10000ppm group, which were compared to control groups, 15% in males and 18% in females.

Histopathology (Table 2 – 5)

Oral cavity

-Male-

Five squamous cell carcinomas and 2 squamous cell papillomas occurred in the 10000ppm group. In the incidence of squamous cell carcinoma and combined incidence of squamous cell carcinoma and papilloma, a positive trend was indicated by Peto test and Cochran-Armitage test, and a significant difference between 10000ppm group and control group by Fisher exact test. Basal cell activation (2/50) was also observed in the 10000ppm group.

-Female-

Squamous cell carcinomas (Control: 0/50, 400ppm: 1/50, 2000ppm: 1/50, 10000ppm: 3/50) occurred at positive trend (Peto test) in the treated groups. Epithelial dysplasia (2/50) and basal cell activation (1/50) were also observed in the 10000ppm group.

Esophagus

-Male-

Squamous cell hyperplasia (1/50) was observed in the 10000ppm group.

-Female-

One squamous cell carcinoma was observed in the 10000ppm group. Squamous cell hyperplasia (1/50) and basal cell activation (4/50) were also observed in the 10000ppm group.

Stomach

-Male-

In the treated groups, basal cell activation (2/50) was observed in the 10000ppm group, and squamous cell hyperplasia (1/50) in the 2000ppm group. In the control group, one squamous cell papilloma and squamous cell hyperplasia (2/50) were observed.

-Female-

Basal cell activation (5/50) was observed in the 10000ppm group.

2) Mouse study

Survival (Fig. 9, 10): There were no significant difference in survival rates at the end of the study between treated groups and control group in both sexes (male; control: 35/50, 400 ppm: 42/50, 2000 ppm: 38/50, 10000 ppm: 33/50, female; control: 26/50, 400 ppm: 27/50, 2000 ppm: 25/50, 10000 ppm: 23/50).

Clinical observation: Some mice of the 10000 ppm group had nodules in the oral cavity (6/50 males and 6/49 females). There were no other abnormal clinical signs in the treated males and females.

Body weight (Fig. 11, 12): At the end of the study, body weight in males and females of the 10000ppm groups were significantly less (30% and 18%, respectively) than controls.

Food and water consumption (Fig. 13 – 16): Food consumption did not show significant variation among groups for both males and females. Water consumption was significantly less than control group in males and females of the 10000ppm group, especially in the latter period of this experiment.

Histopathology (Table 6 – 12)

Oral cavity

-Male-

Thirteen squamous cell carcinomas and 4 squamous cell papillomas occurred in the 10000ppm group. In the incidence of squamous cell carcinoma, squamous cell papilloma and combined incidence of squamous cell carcinoma and papilloma, Peto test and Cochran-Armitage test indicated a positive trend. And a significant difference between 10000ppm group and control group was indicated in the incidence of squamous cell carcinoma and combined incidence of squamous cell carcinoma and papilloma by Fisher exact test. Squamous cell hyperplasia (2000ppm: 2/50, 10000ppm: 13/50), basal cell activation (2000ppm: 1/50, 10000ppm: 18/50) and epithelial dysplasia (10000ppm: 24/50) were also observed in the treated groups.

-Female-

Fifteen squamous cell carcinomas and 3 squamous cell papillomas occurred in the 10000ppm group. In the incidence of squamous cell carcinoma, squamous cell papilloma and combined incidence of squamous cell carcinoma and papilloma, Peto test and Cochran-Armitage test indicated a positive trend. And a significant difference between 10000ppm group and control group was indicated in the incidence of squamous cell carcinoma and combined incidence of squamous cell carcinoma and papilloma by Fisher exact test. Squamous cell hyperplasia (2000ppm: 1/50, 10000ppm: 6/49), basal cell activation (2000ppm: 1/50, 10000ppm: 17/49) and epithelial dysplasia (10000ppm: 17/49) were also observed in the treated groups.

Esophagus

-Male-

Seven squamous cell carcinomas occurred in the 10000ppm group. In the incidence of squamous cell carcinoma, a positive trend was indicated by Peto test and Cochran-Armitage test, and a significant difference between 10000ppm group and control group by Fisher exact test. Squamous cell hyperplasia (2/50), basal cell activation (9/50) and epithelial dysplasia (10000ppm: 2/50) were also observed in the 10000ppm group.

-Female-

Squamous cell carcinoma (10000ppm: 1/49) and Squamous cell papilloma (2000ppm: 1/50) were observed in the treated groups. Squamous cell hyperplasia (2/49), basal cell activation (15/49) and epithelial dysplasia (7/49) were also observed in the 10000ppm group.

Stomach

-Male-

Seven squamous cell carcinomas and 2 squamous cell papillomas occurred in the 10000ppm group, and one squamous cell carcinoma in the control group. In the incidence of squamous cell carcinoma and combined incidence of squamous cell carcinoma and papilloma, a positive trend was indicated by Peto test and Cochran-Armitage test, and significant difference between 10000ppm group and control group by Fisher exact test. Squamous cell hyperplasia (3/50), basal cell activation (1/50) and epithelial dysplasia (1/50) were also observed in the 10000ppm group.

-Female-

Three squamous cell carcinomas and 1 squamous cell papilloma occurred in the 10000ppm group. In the incidence of squamous cell carcinoma and combined incidence of squamous cell carcinoma and papilloma, Peto test and Cochran-Armitage test indicated a positive trend. And a significant difference between 10000ppm group and control group was indicated in the combined incidence of squamous cell carcinoma and papilloma by Fisher exact test. Squamous cell hyperplasia (400ppm: 2/50, 10000ppm: 4/49) and basal cell activation (10000ppm: 1/49) were also observed in the treated groups.

Larynx

-Male-

Two squamous cell carcinomas occurred in the 10000ppm group. Squamous cell hyperplasia (1/50), basal cell activation (3/50) and epithelial dysplasia (2/50) were also observed in the 10000ppm group.

-Female-

Squamous cell carcinomas (2000ppm: 1/50, 10000ppm: 1/49) were observed in the treated groups. Basal cell activation (6/49) and epithelial dysplasia (3/49) were also observed in the 10000ppm group.

3. Conclusions

Under the conditions of these 2-year drinking water study studies, there was clear evidence of carcinogenic activity of vinyl acetate for male and female F344/DuCrj rats (Fischer), as indicated by increased incidences of squamous cell carcinoma / papilloma of the oral cavity in males, and squamous cell carcinoma of the oral cavity and esophagus in females.

For male and female Crj:BDF1 mice, there was clear evidence of carcinogenic activity of vinyl acetate, as indicated by increased incidences of squamous cell carcinoma /

papilloma of the oral cavity and stomach and squamous cell carcinoma of the esophagus and larynx in males, and squamous cell carcinoma / papilloma of the oral cavity, stomach and esophagus and squamous cell carcinoma of the larynx in females.

These studies have been carried out by the Japan Bioassay Research Center under contract with the Ministry of Labour of Japan (the Ministry of Labour has reorganized to the Ministry of Health, Labour and Welfare in January 2001), and final report has been submitted to the Ministry of Labour in 1995.

TABLE 1 EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE DRINKING WATER STUDIES OF VINYL ACETATE

Two-year studies

<Method of Administration>
Drinking water
<Number of Groups>
Male 4, Female 4
<Size of Groups>
50 males and 50 females of each group
<Animals>
Strain and Species
F344/DuCrj(Fischer) rat
Crj:BDF1 mouse
Animal Source
Charles River Japan, Inc.
Duration Held Before Study
2 wk
Age When Placed on Study
6 wk
Age When Killed
110～111 wk
<Doses>
Rat-------<Male> 0, 400, 2000 or 10000 ppm
<Female> 0, 400, 2000 or 10000 ppm
Mouse---<Male> 0, 400, 2000 or 10000 ppm
<Female> 0, 400, 2000 or 10000 ppm
<Duration of Dosing>
7d/wk for 104wk
<Animal Maintenance>
Feed
CRF-1 (Oriental Yeast Co., Ltd.)
Sterilized by γ -ray
Available *ad libitum*
Water
Filtrated and sterilized by ultraviolet ray
Automatic watering system in duration of quarantine
Glass bottle in duration of acclimation and administration
Available *ad libitum*
Animal per Cage
Single (stainless steel wire)
Animal Room Environment
Barrier system
Temperature : 24±2℃
Humidity : 55±10%
Fluorescent light 12h/d
15-17 room air changes /h
<Type and Frequency of Observation>
Clinical Sign
Observed 1 per d
Body Weight
Weighed 1 per wk for 14wk
Weighed 1 per 2wks thereafter
Food Consumption
Weighed 1 per wk for 14wk
Weighed 1 per 4wks thereafter
Water Consumption
Weighed 2 per wk for 14wk
Weighed 1 per 2wks thereafter

TABLE 1 EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE DRINKING WATER STUDIES OF VINYL ACETATE (Continued)

| Two-year studies |
|---|
| <Hematology><br>Red blood cell (RBC),<br>Hemoglobin, Hematocrit,<br>Mean corpuscular volume (MCV),<br>Mean corpuscular hemoglobin (MCH),<br>Mean corpuscular hemoglobin concentration (MCHC),<br>Platelet, White blood cell (WBC),<br>Differential WBC.<br><Biochemistry><br>Total protein, Albumin,<br>A/G ratio, T-bilirubin, Glucose,<br>T-cholesterol, Triglyceride,<br>Phospholipid <rat only>,<br>Glutamic oxaloacetic transaminase (GOT),<br>Glutamic pyruvic transaminase (GPT),<br>Lactate dehydrogenase (LDH),<br>Alkaline phosphatase (ALP),<br>γ -Glutamyl transpeptidase (G-GTP) <rat only>,<br>Creatine phosphokinase (CPK), Urea nitrogen,<br>Creatinine <rat only>,<br>Sodium, Potassium, Chloride,<br>Calcium, Inorganic phosphorus.<br><Urinalysis><br>pH, Protein, Glucose, Ketone body<br>Bilirubin <rat only>, Occult blood<br>Urobilinogen.<br><Necropsy><br>Necropsy performed on all animals.<br><Organ weight><br>Organ weight measurement performed on scheduled sacrificed animals.<br>The following organs were weighed:<br>brain, lung, liver, spleen, heart, kidney, adrenal, testis, ovary.<br><Histopathologic Examination><br>Histopathologic examination performed on all animals.<br>The following organs were examined:<br>skin, nasal cavity, trachea, lung, bone marrow, lymph node, thymus, spleen, heart, tongue, salivary gland, esophagus, stomach, small intestine, large intestine, liver, pancreas, kidney, urinary bladder, pituitary, thyroid, adrenal, testis, epididymis, seminal vesicle, prostate, ovary, uterus, vagina, mammary gland, brain, spinal cord, peripheral nerve, eye, Harderian gland, muscle, bone. |

TABLE 2 SELECTED LESIONS OF DIGESTIVE SYSTEM IN MALE RATS

| | neoplastic lesions | | | | | | | | non neoplastic lesions | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | squamous cell papilloma | | | | squamous cell carcinoma | | | | squamous cell hyperplasia | | | | basal cell activation | | | | epithelial dysplasia | | | |
| Group(ppm) | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 |
| Number of animals | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| oral cavity | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| esophagus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| stomach | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |

TABLE 3 SELECTED LESIONS OF DIGESTIVE SYSTEM IN FEMALE RATS

| | neoplastic lesions | | | | | | | | non neoplastic lesions | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | squamous cell papilloma | | | | squamous cell carcinoma | | | | squamous cell hyperplasia | | | | basal cell activation | | | | epithelial dysplasia | | | |
| Group(ppm) | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 |
| Number of animals | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| oral cavity | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 |
| esophagus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 |
| stomach | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |

TABLE 4 NEOPLASTIC LESIONS (ORAL CAVITY) INCIDENCE AND STATISTICAL ANALYSIS IN MALE RATS

| Group Name | Control | 400 ppm | 2000 ppm | 10000 ppm |
|---|---|---|---|---|
| SITE : oral cavity | | | | |
| TUMOUR : squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 5/50 (10.0) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 7.69 |
| Terminal Rates(c) | 0/44 ( 0.0) | 0/40 ( 0.0) | 0/36 ( 0.0) | 3/39 ( 7.7) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.0161* ? | | | |
| Prevalence Method(d) | P=0.0019**? | | | |
| Combined analysis(d) | P=0.0001**? | | | |
| Cochran-Armitage Test(e) | P=0.0001** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.0360* |
| SITE : oral cavity | | | | |
| TUMOUR : squamous cell papilloma, squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 7/50 (14.0) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 12.82 |
| Terminal Rates(c) | 0/44 ( 0.0) | 0/40 ( 0.0) | 0/36 ( 0.0) | 5/39 (12.8) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.0161* ? | | | |
| Prevalence Method(d) | P<0.0001**? | | | |
| Combined analysis(d) | P<0.0001**? | | | |
| Cochran-Armitage Test(e) | P<0.0001**? | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.0101* |

TABLE 5 NEOPLASTIC LESIONS (ORAL CAVITY) INCIDENCE AND STATISTICAL ANALYSIS IN FEMALE RATS

| Group Name | Control | 400 ppm | 2000 ppm | 10000 ppm |
|---|---|---|---|---|
| SITE : oral cavity | | | | |
| TUMOUR : squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 1/50 ( 2.0) | 1/50 ( 2.0) | 3/50 ( 6.0) |
| Adjusted Rates(b) | 0.0 | 2.50 | 2.44 | 8.11 |
| Terminal Rates(c) | 0/41 ( 0.0) | 1/40 ( 2.5) | 1/41 ( 2.4) | 3/37 ( 8.1) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=------ | | | |
| Prevalence Method(d) | P=0.0342* | | | |
| Combined analysis(d) | P=------ | | | |
| Cochran-Armitage Test(e) | P=0.0590 | | | |
| Fisher Exact Test(e) | | P=0.4950 | P=0.4950 | P=0.1325 |

(a):Number of tumor-bearing animals/number of animals examined at the site.
(b):Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c):Observed tumor incidence at terminal kill.
(d):Beneth the control incidence are the P-values associated with the trend test.
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combined analysis : Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates.
------: There is no data which should be statistical analysis.
Significant difference : * : $P \leq 0.05$ ** : $P \leq 0.01$

TABLE 6 SELECTED LESIONS OF DIGESTIVE SYSTEM AND LARYNX IN MALE MICE

| | neoplastic lesions | | | | | | | | non neoplastic lesions | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | squamous cell papilloma | | | | squamous cell carcinoma | | | | squamous cell hyperplasia | | | | basal cell activation | | | | epithelial dysplasia | | | |
| Group(ppm) | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 |
| Number of animals | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| oral cavity | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 13 | 0 | 0 | 2 | 13 | 0 | 0 | 1 | 18 | 0 | 0 | 0 | 24 |
| esophagus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 2 |
| stomach | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 7 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| larynx | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 2 |

TABLE 7 SELECTED LESIONS OF DIGESTIVE SYSTEM AND LARYNX IN FEMALE MICE

| | neoplastic lesions | | | | | | | | non neoplastic lesions | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | squamous cell papilloma | | | | squamous cell carcinoma | | | | squamous cell hyperplasia | | | | basal cell activation | | | | epithelial dysplasia | | | |
| Group(ppm) | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 | 0 | 400 | 2000 | 10000 |
| Number of animals | 50 | 50 | 50 | 49 | 50 | 50 | 50 | 49 | 50 | 50 | 50 | 49 | 50 | 50 | 50 | 49 | 50 | 50 | 50 | 49 |
| oral cavity | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 15 | 0 | 0 | 1 | 6 | 0 | 0 | 1 | 17 | 0 | 0 | 0 | 17 |
| esophagus | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 7 |
| stomach | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 3 | 0 | 2 | 0 | 4 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| larynx | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 3 |

TABLE 8 NEOPLASTIC LESIONS (ORAL CAVITY) INCIDENCE AND STATISTICAL ANALYSIS IN MALE MICE

| Group Name | Control | 400 ppm | 2000 ppm | 10000 ppm |
|---|---|---|---|---|
| SITE : oral cavity | | | | |
| TUMOUR : squamous cell papilloma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 4/50 (10.0) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 9.76 |
| Terminal Rates(c) | 0/35 ( 0.0) | 0/42 ( 0.0) | 0/38 ( 0.0) | 3/33 ( 9.1) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=------ | | | |
| Prevalence Method(d) | P=0.0003**? | | | |
| Combined analysis(d) | P=------ | | | |
| Cochran-Armitage Test(e) | P=0.0006** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.0688 |
| SITE : oral cavity | | | | |
| TUMOUR : squamous cell carcinnoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 13/50 (26.0) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 17.07 |
| Terminal Rates(c) | 0/35 ( 0.0) | 0/42 ( 0.0) | 0/38 ( 0.0) | 4/33 (12.1) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P<0.0001**? | | | |
| Prevalence Method(d) | P<0.0001**? | | | |
| Combined analysis(d) | P<0.0001**? | | | |
| Cochran-Armitage Test(e) | P<0.0001** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.0003** |
| SITE : oral cavity | | | | |
| TUMOUR : squamous cell papilloma, squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 16/50 (32.0) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 24.39 |
| Terminal Rates(c) | 0/35 ( 0.0) | 0/42 ( 0.0) | 0/33 ( 0.0) | 7/33 (21.2) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P<0.0001**? | | | |
| Prevalence Method(d) | P<0.0001**? | | | |
| Combined analysis(d) | P<0.0001**? | | | |
| Cochran-Armitage Test(e) | P<0.0001** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P<0.0001** |

(a):Number of tumor-bearing animals/number of animals examined at the site.
(b):Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c):Observed tumor incidence at terminal kill.
(d):Beneth the control incidence are the P-values associated with the trend test.
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combined analysis : Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates.
? :The conditional probabilities of the largest and smallest possible out comes can not estimated or this P-value isyond the estimated P-value.
------: There is no data which should be statistical analysis.
Significant difference ; * : $P \leq 0.05$ ** : $P \leq 0.01$

TABLE 9 NEOPLASTIC LESIONS (ORAL CAVITY) INCIDENCE AND STATISTICAL ANALYSIS IN FEMALE MICE

| Group Name | Control | 400 ppm | 2000 ppm | 10000 ppm |
|---|---|---|---|---|
| SITE : oral cavity | | | | |
| TUMOUR : squamous cell papilloma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 3/49 ( 6.1) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 12.50 |
| Terminal Rates(c) | 0/26 ( 0.0) | 0/27 ( 0.0) | 0/25 ( 0.0) | 2/23 ( 8.7) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=------ | | | |
| Prevalence Method(d) | P=0.0014**? | | | |
| Combined analysis(d) | P=------ | | | |
| Cochran-Armitage Test(e) | P=0.0027** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.1287 |
| SITE : oral cavity | | | | |
| TUMOUR : squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 15/49 (30.6) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 35.48 |
| Terminal Rates(c) | 0/26 ( 0.0) | 0/27 ( 0.0) | 0/25 ( 0.0) | 8/23 (34.8) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.0004**? | | | |
| Prevalence Method(d) | P<0.0001**? | | | |
| Combined analysis(d) | P<0.0001**? | | | |
| Cochran-Armitage Test(e) | P<0.0001** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.0001** |
| SITE : oral cavity | | | | |
| TUMOUR : squamous cell papilloma, squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 18/49 (36.7) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 45.83 |
| Terminal Rates(c) | 0/26 ( 0.0) | 0/27 ( 0.0) | 0/25 ( 0.0) | 7/23 (43.5) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.0004**? | | | |
| Prevalence Method(d) | P<0.0001**? | | | |
| Combined analysis(d) | P<0.0001**? | | | |
| Cochran-Armitage Test(e) | P<0.0001** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P<0.0001** |

(a):Number of tumor-bearing animals/number of animals examined at the site.
(b):Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c):Observed tumor incidence at terminal kill.
(d):Beneth the control incidence are the P-values associated with the trend test.
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combined analysis : Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates.
? :The conditional probabilities of the largest and smallest possible out comes can not estimated or this P-value isyond the estimated P-value.
------: There is no data which should be statistical analysis.
Significant difference ; * : $P \leq 0.05$ ** : $P \leq 0.01$

TABLE 10 NEOPLASTIC LESIONS (ESOPHAGUS) INCIDENCE AND STATISTICAL ANALYSIS IN MALE MICE

| Group Name | Control | 400 ppm | 2000 ppm | 10000 ppm |
|---|---|---|---|---|
| SITE : esophagus | | | | |
| TUMOUR : squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 7/50 (14.0) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 15.15 |
| Terminal Rates(c) | 0/35 ( 0.0) | 0/42 ( 0.0) | 0/38 ( 0.0) | 5/33 (15.2) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.1801 | | | |
| Prevalence Method(d) | P<0.0001**? | | | |
| Combined analysis(d) | P<0.0001**? | | | |
| Cochran-Armitage Test(e) | P<0.0001** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.0101* |

(a):Number of tumor-bearing animals/number of animals examined at the site.
(b):Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c):Observed tumor incidence at terminal kill.
(d):Beneth the control incidence are the P-values associated with the trend test.
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combined analysis : Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates.
? :The conditional probabilities of the largest and smallest possible out comes can not estimated or this P-value beyond is the estimated P-value.
Significant difference : * : $P \leq 0.05$ ** : $P \leq 0.01$

TABLE 11 NEOPLASTIC LESIONS (STOMACH) INCIDENCE AND STATISTICAL ANALYSIS MALE MICE

| Group Name | Control | 400 ppm | 2000 ppm | 10000 ppm |
|---|---|---|---|---|
| SITE : stomach | | | | |
| TUMOUR : squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 1/50 ( 2.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 7/50 (14.0) |
| Adjusted Rates(b) | 2.86 | 0.0 | 0.0 | 18.18 |
| Terminal Rates(c) | 1/35 ( 2.9) | 0/42 ( 0.0) | 0/38 ( 0.0) | 6/33 (18.2) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.1821 | | | |
| Prevalence Method(d) | P=0.0001** | | | |
| Combined analysis(d) | P<0.0001** | | | |
| Cochran-Armitage Test(e) | P=0.0001** | | | |
| Fisher Exact Test(e) | | P=0.4950 | P=0.4950 | P=0.0430* |
| SITE : stomach | | | | |
| TUMOUR : squamous cell papilloma, squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 1/50 ( 2.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 9/50 (18.0) |
| Adjusted Rates(b) | 2.86 | 0.0 | 0.0 | 24.24 |
| Terminal Rates(c) | 1/35 ( 2.9) | 0/42 ( 0.0) | 0/38 ( 0.0) | 8/33 (24.2) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.1821 | | | |
| Prevalence Method(d) | P=0.0001** | | | |
| Combined analysis(d) | P<0.0001** | | | |
| Cochran-Armitage Test(e) | P=0.0001** | | | |
| Fisher Exact Test(e) | | P=0.4950 | P=0.4950 | P=0.0150* |

(a):Number of tumor-bearing animals/number of animals examined at the site.
(b):Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c):Observed tumor incidence at terminal kill.
(d):Beneth the control incidence are the P-values associated with the trend test.
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combined analysis : Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates.
Significant difference ; * : $P \leq 0.05$ ** : $P \leq 0.01$

TABLE 12 NEOPLASTIC LESIONS (STOMACH) INCIDENCE AND STATISTICAL ANALYSIS IN FEMALE MICE

| Group Name | Control | 400 ppm | 2000 ppm | 10000 ppm |
|---|---|---|---|---|
| SITE : stomach | | | | |
| TUMOUR : squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 3/49 ( 6.1) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 4.35 |
| Terminal Rates(c) | 0/26 ( 0.0) | 0/27 ( 0.0) | 0/25 ( 0.0) | 1/23 ( 4.3) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.0146* ? | | | |
| Prevalence Method(d) | P=0.1561 | | | |
| Combined analysis(d) | P=0.0019**? | | | |
| Cochran-Armitage Test(e) | P=0.0027** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.1287 |
| SITE : stomach | | | | |
| TUMOUR : squamous cell papilloma, squamous cell carcinoma | | | | |
| Tumor Rates | | | | |
| Overall Rates(a) | 0/50 ( 0.0) | 0/50 ( 0.0) | 0/50 ( 0.0) | 4/49 ( 8.2) |
| Adjusted Rates(b) | 0.0 | 0.0 | 0.0 | 8.70 |
| Terminal Rates(c) | 0/26 ( 0.0) | 0/27 ( 0.0) | 0/25 ( 0.0) | 2/23 ( 8.7) |
| Statistical Analysis | | | | |
| Peto Test | | | | |
| Standard Method(d) | P=0.0146* ? | | | |
| Prevalence Method(d) | P=0.0103* ? | | | |
| Combined analysis(d) | P=0.0002**? | | | |
| Cochran-Armitage Test(e) | P=0.0005** | | | |
| Fisher Exact Test(e) | | P=0.5000 | P=0.5000 | P=0.0662** |

(a):Number of tumor-bearing animals/number of animals examined at the site.
(b):Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c):Observed tumor incidence at terminal kill.
(d):Beneth the control incidence are the P-values associated with the trend test.
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combined analysis : Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates.
? :The conditional probabilities of the largest and smallest possible out comes can not estimated or this P-value beyond is the estimated P-value.
Significant difference ; * : $P \leqq 0.05$ ** : $P \leqq 0.01$

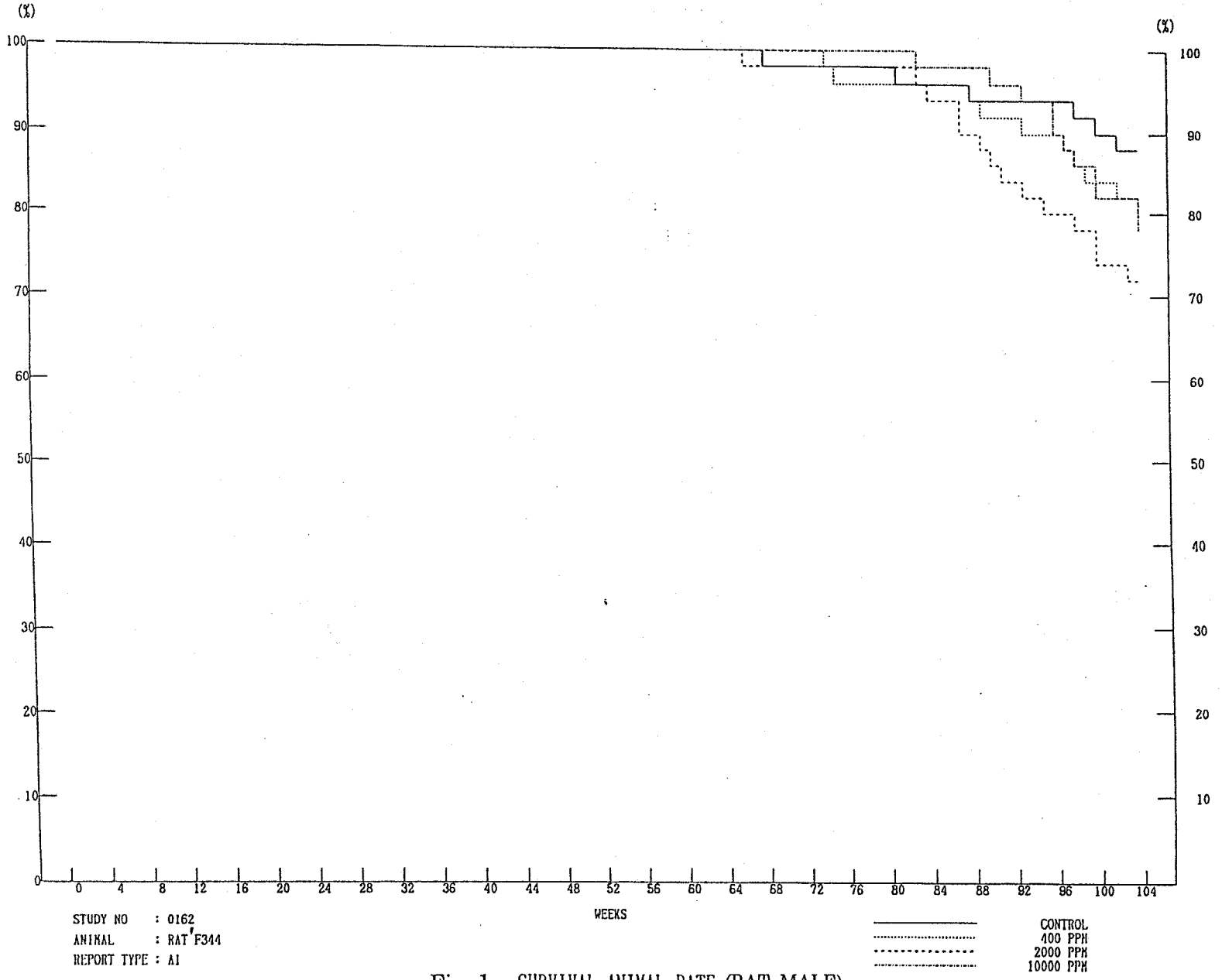


Fig. 1 SURVIVAL ANIMAL RATE (RAT, MALE)

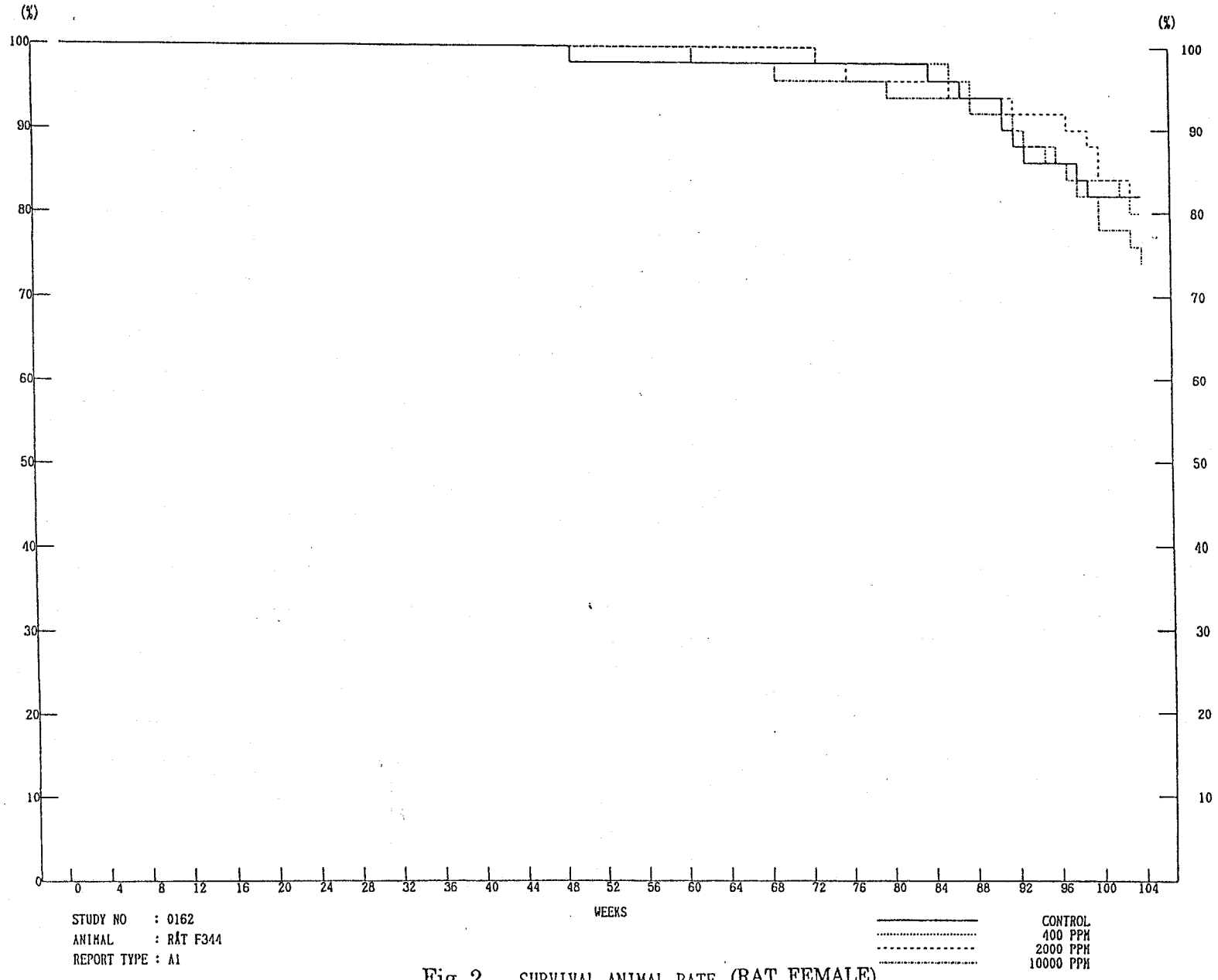


Fig. 2 SURVIVAL ANIMAL RATE (RAT, FEMALE)

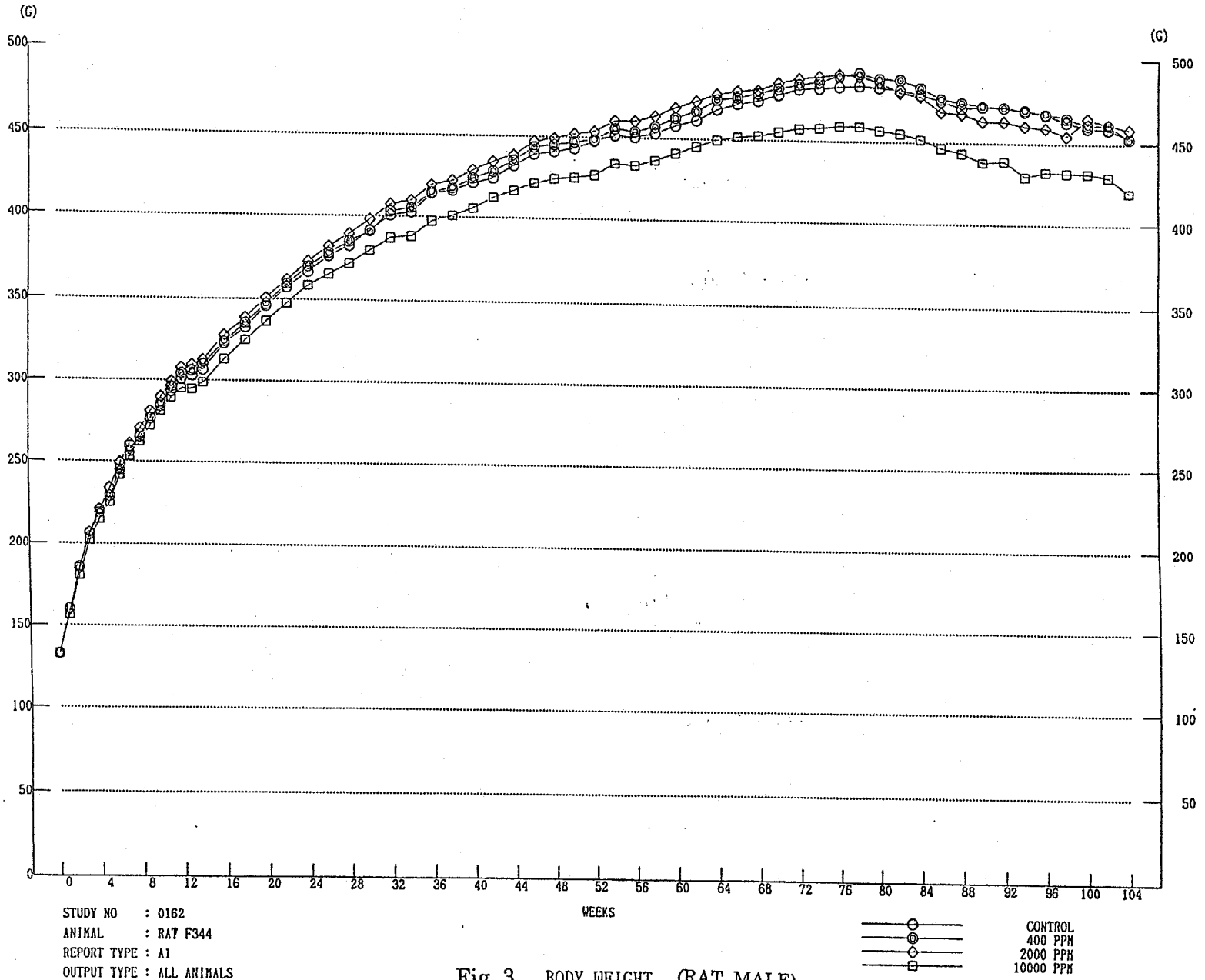


Fig. 3 BODY WEIGHT (RAT, MALE)

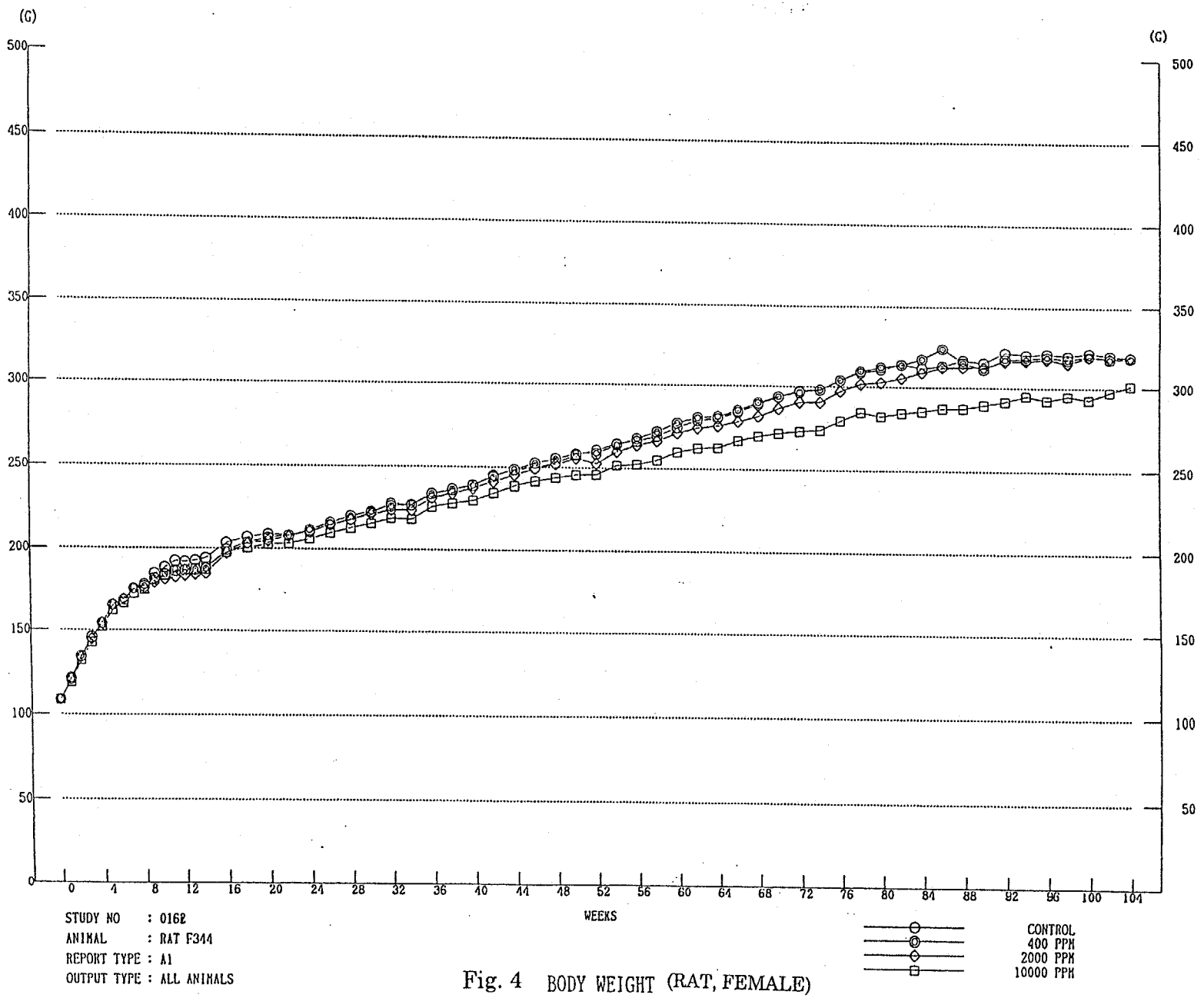


Fig. 4 BODY WEIGHT (RAT, FEMALE)

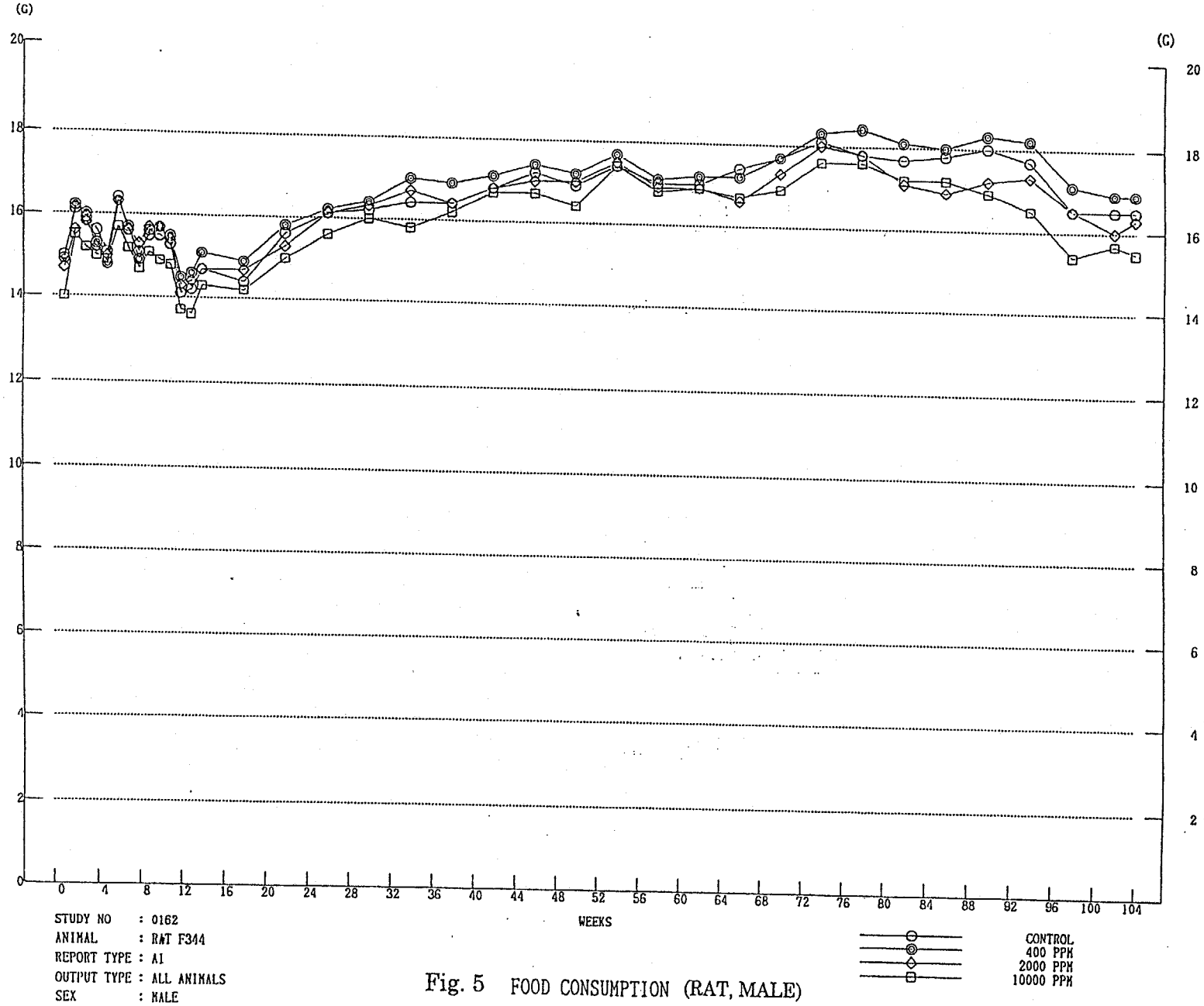


Fig. 5 FOOD CONSUMPTION (RAT, MALE)

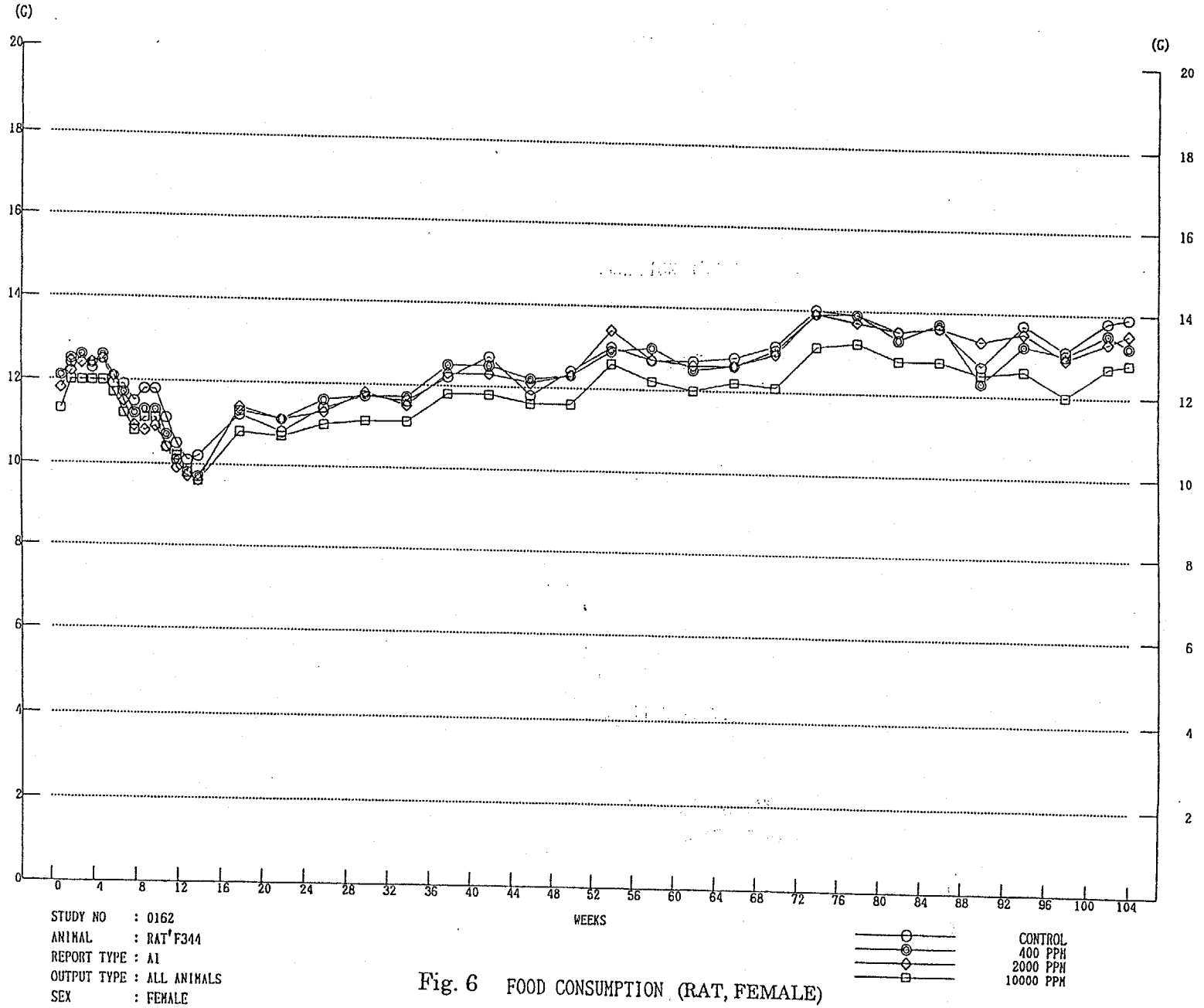


Fig. 6 FOOD CONSUMPTION (RAT, FEMALE)

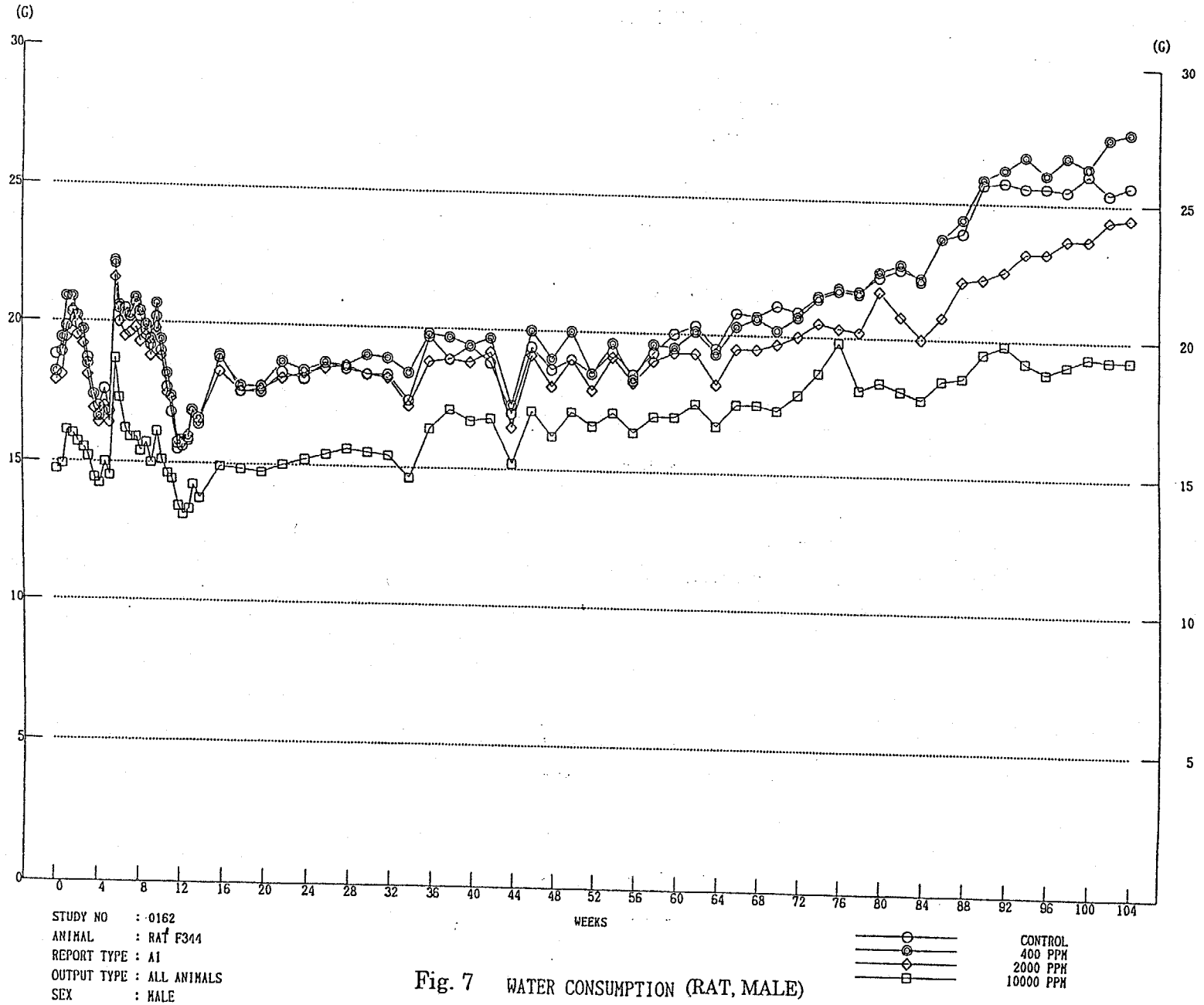


Fig. 7 WATER CONSUMPTION (RAT, MALE)

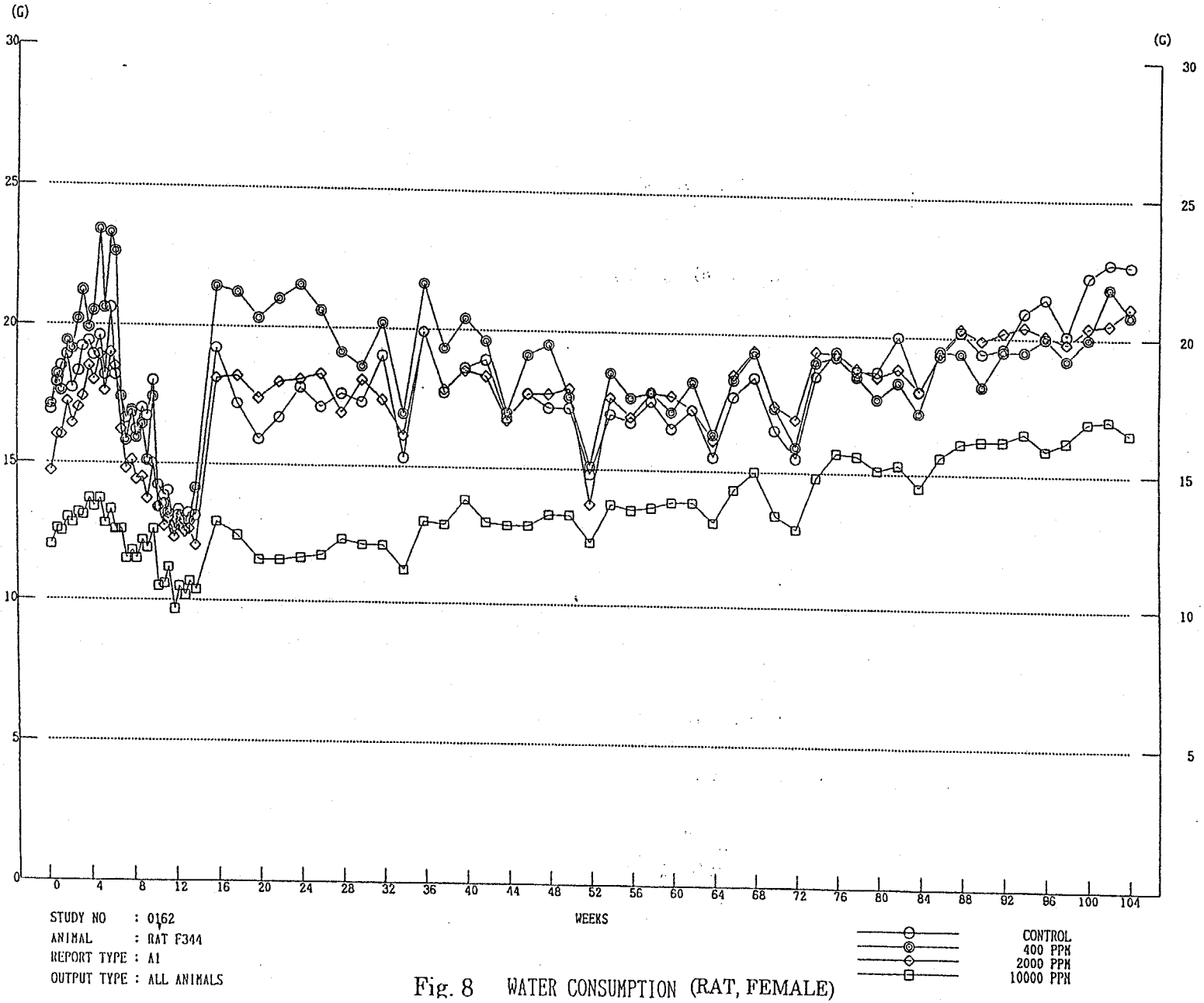


Fig. 8 WATER CONSUMPTION (RAT, FEMALE)

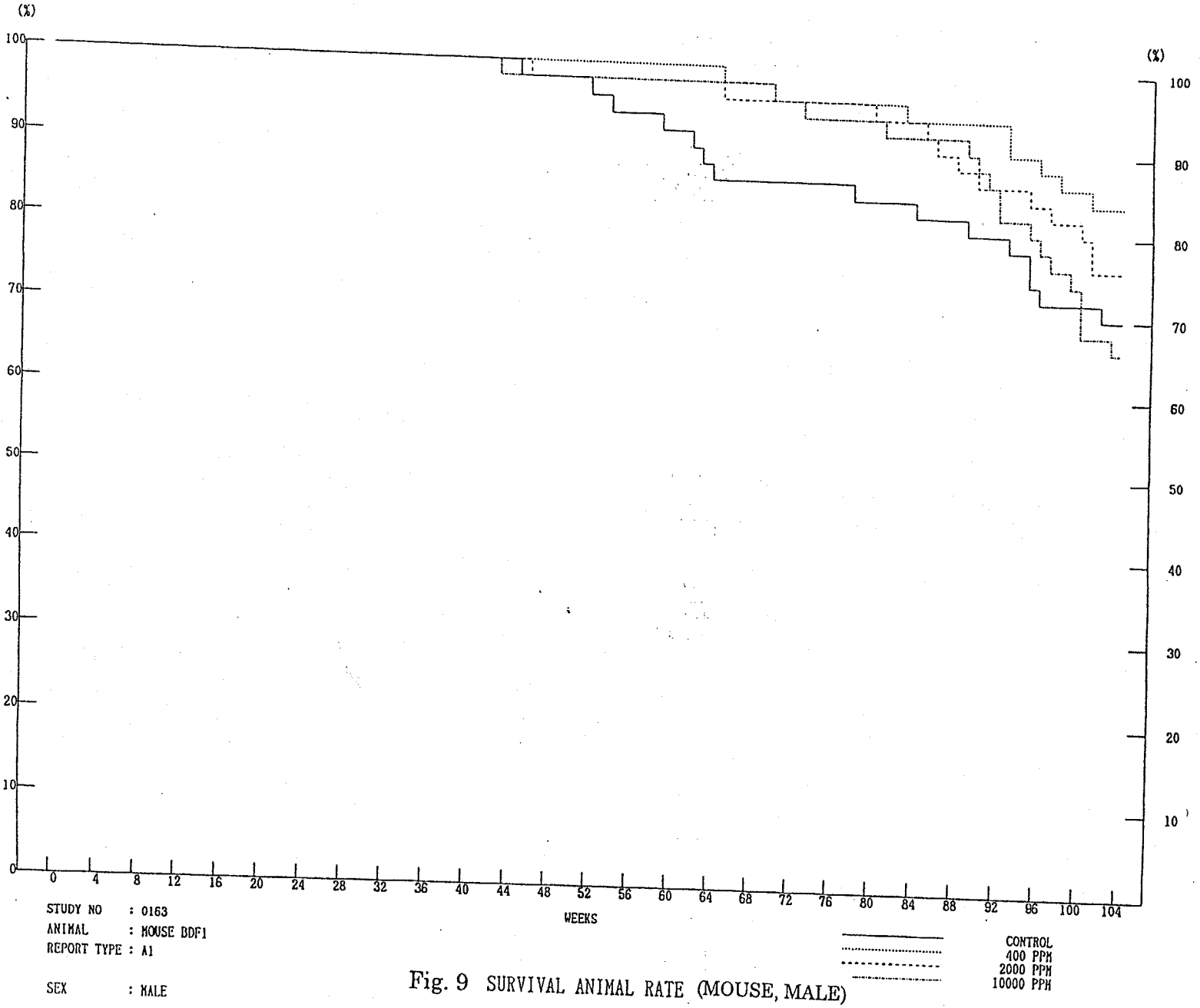


Fig. 9 SURVIVAL ANIMAL RATE (MOUSE, MALE)

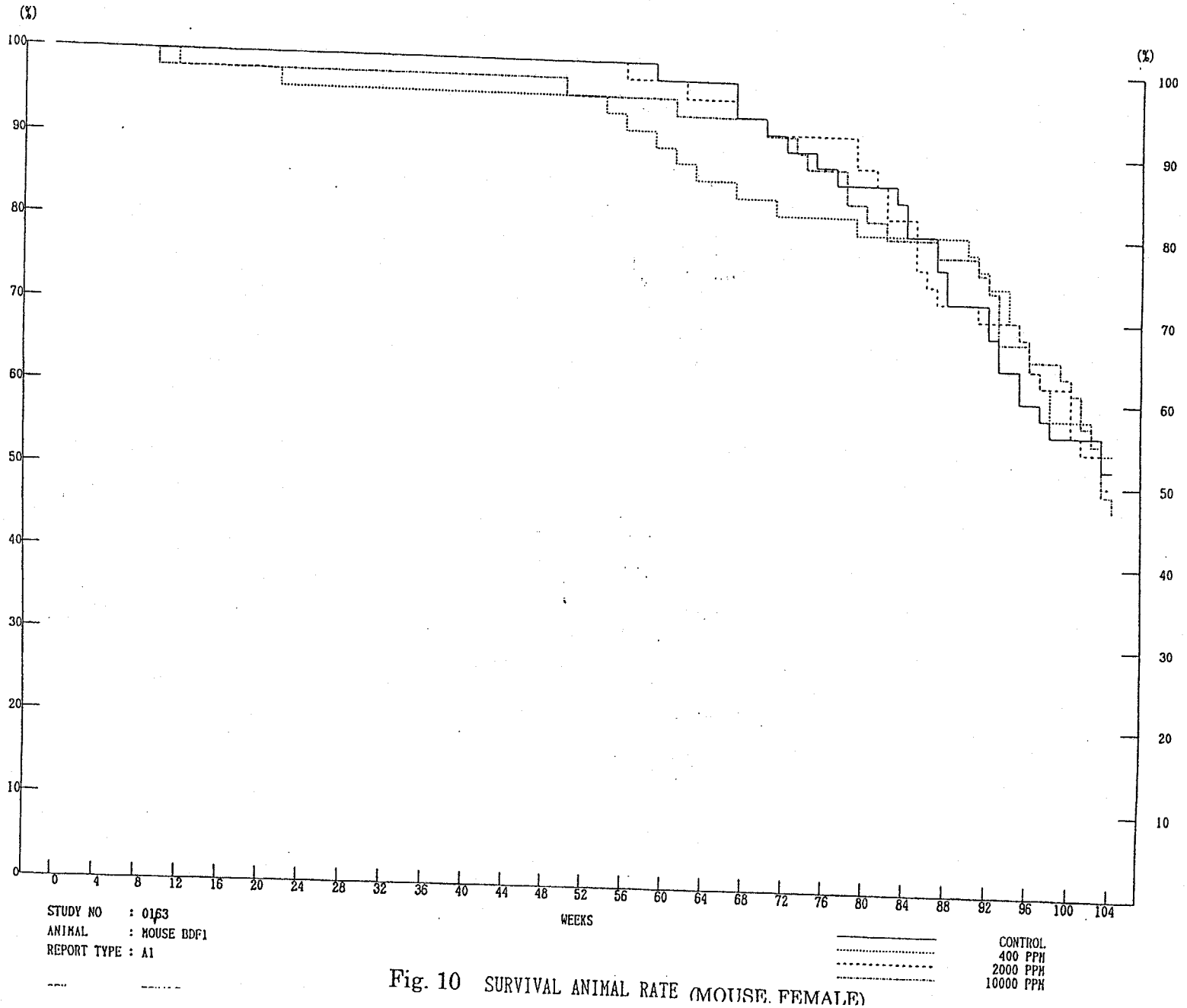


Fig. 10 SURVIVAL ANIMAL RATE (MOUSE, FEMALE)

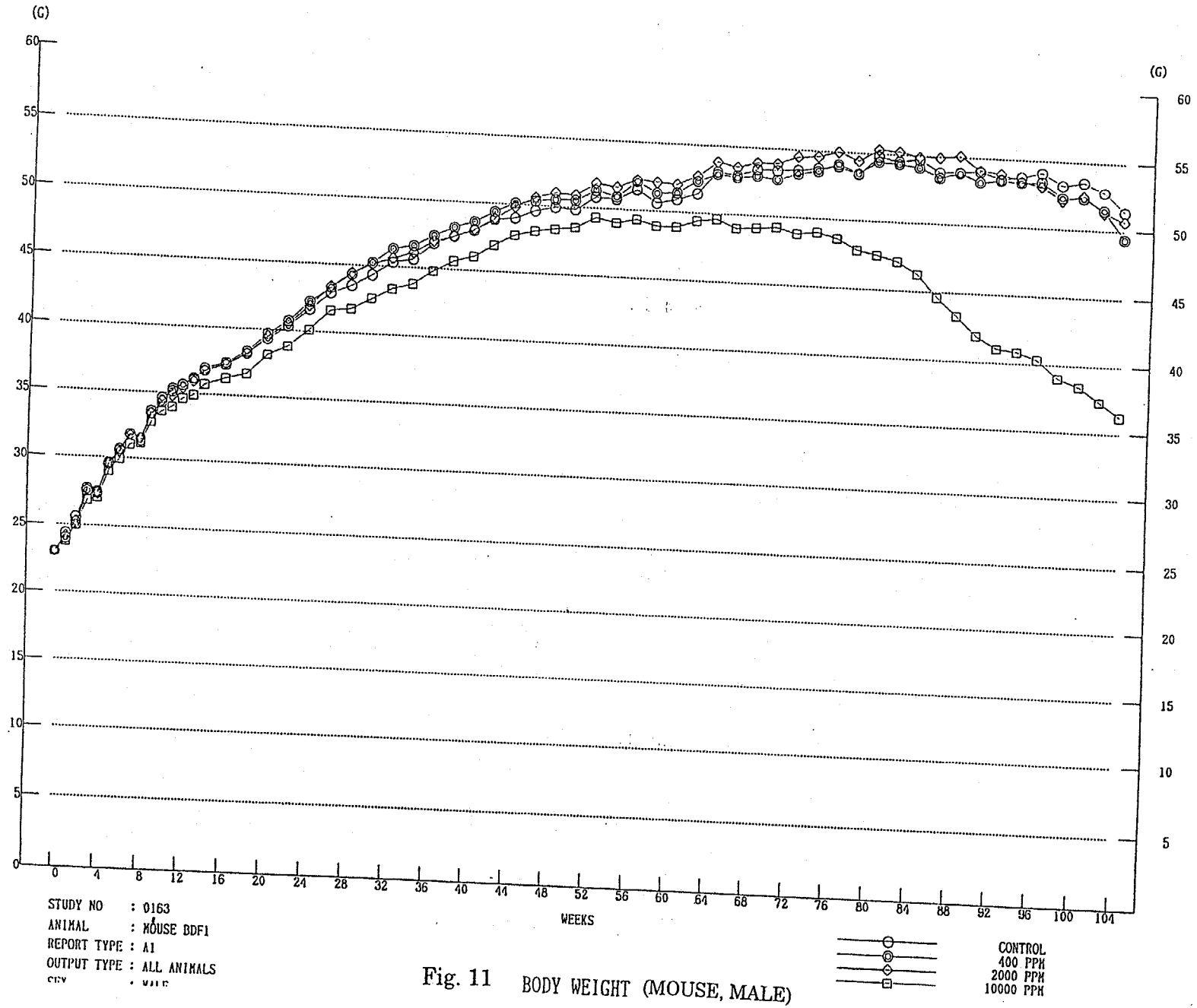


Fig. 11 BODY WEIGHT (MOUSE, MALE)

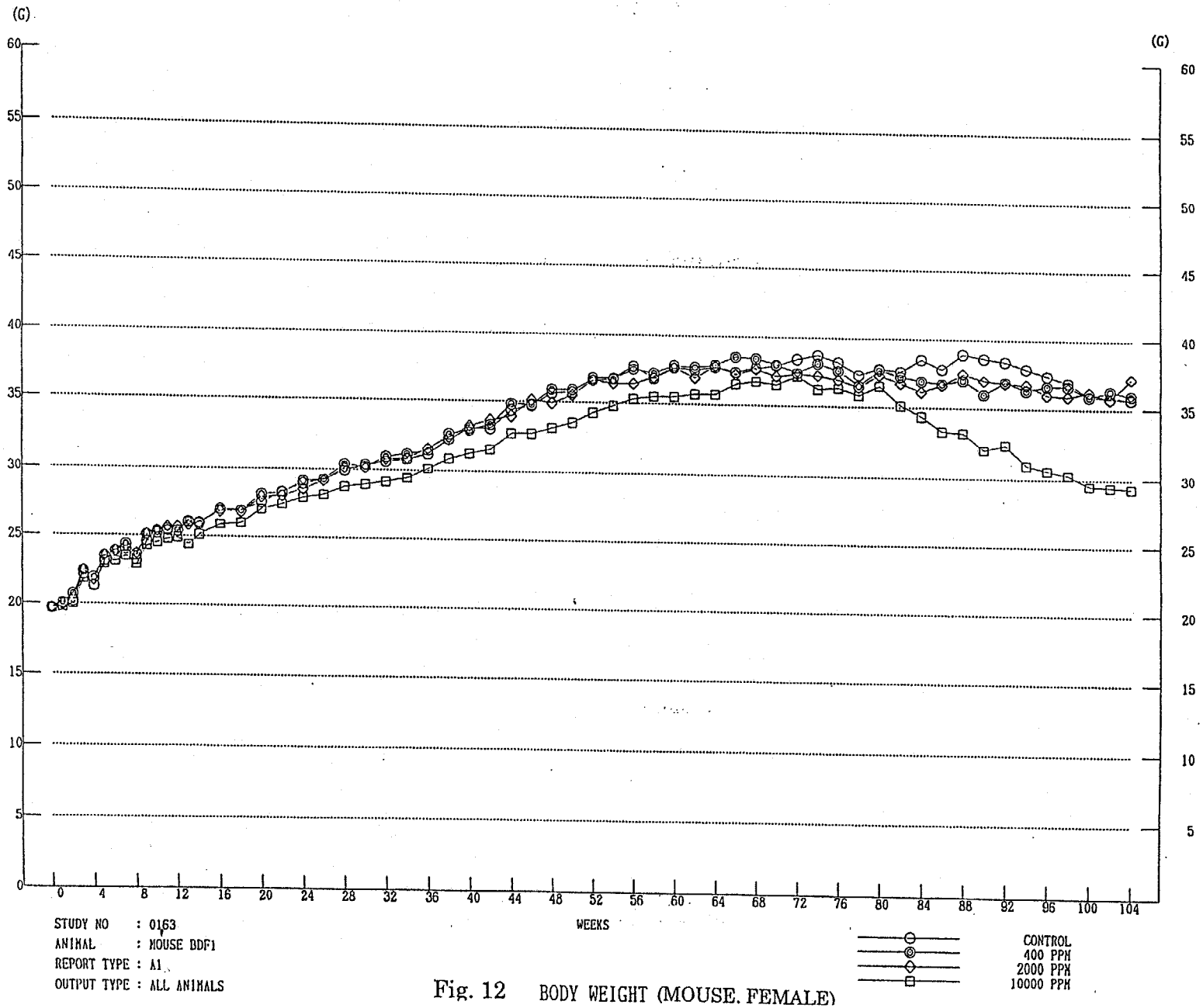


Fig. 12 BODY WEIGHT (MOUSE. FEMALE)

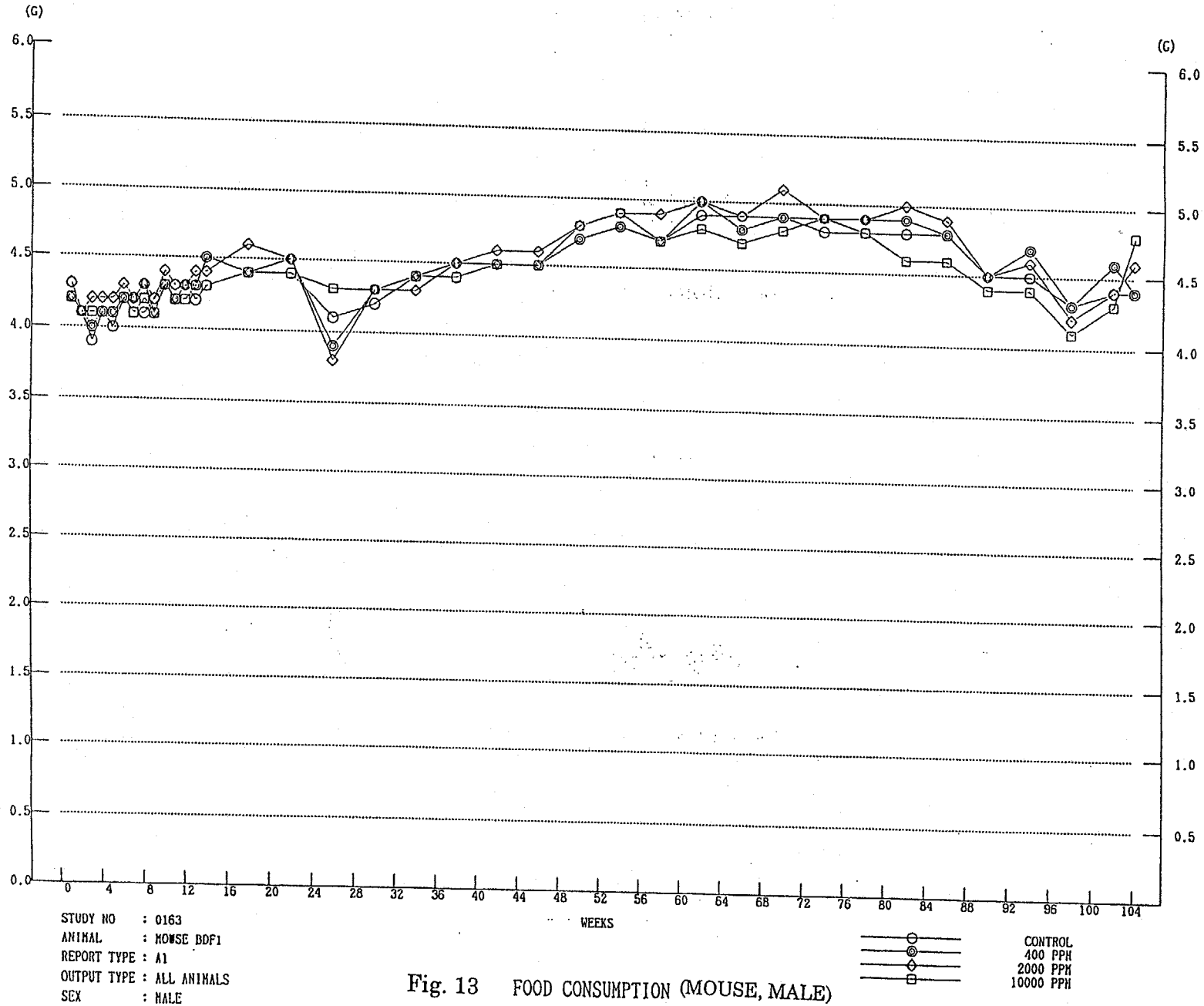


Fig. 13 FOOD CONSUMPTION (MOUSE, MALE)

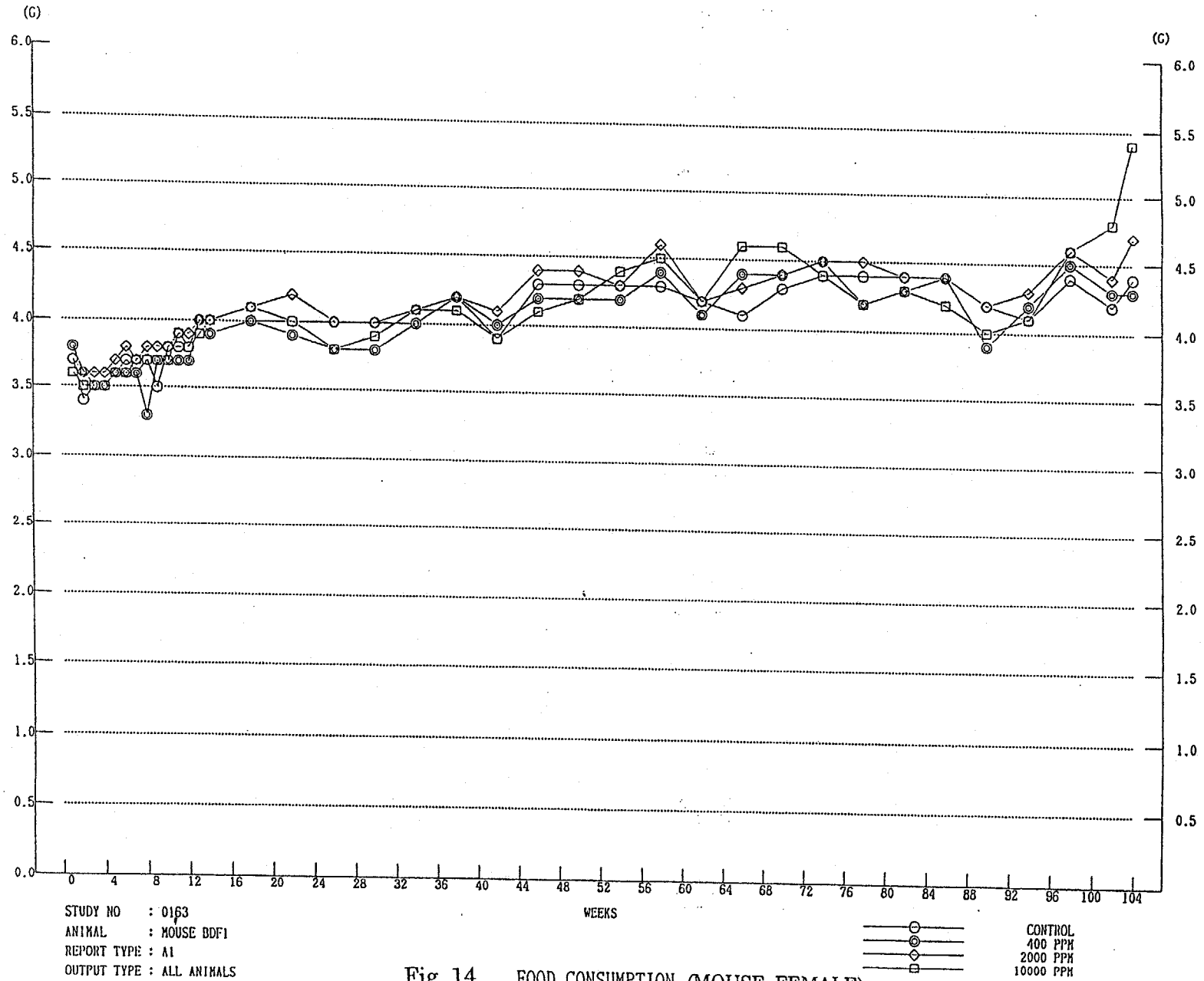


Fig. 14 FOOD CONSUMPTION (MOUSE, FEMALE)

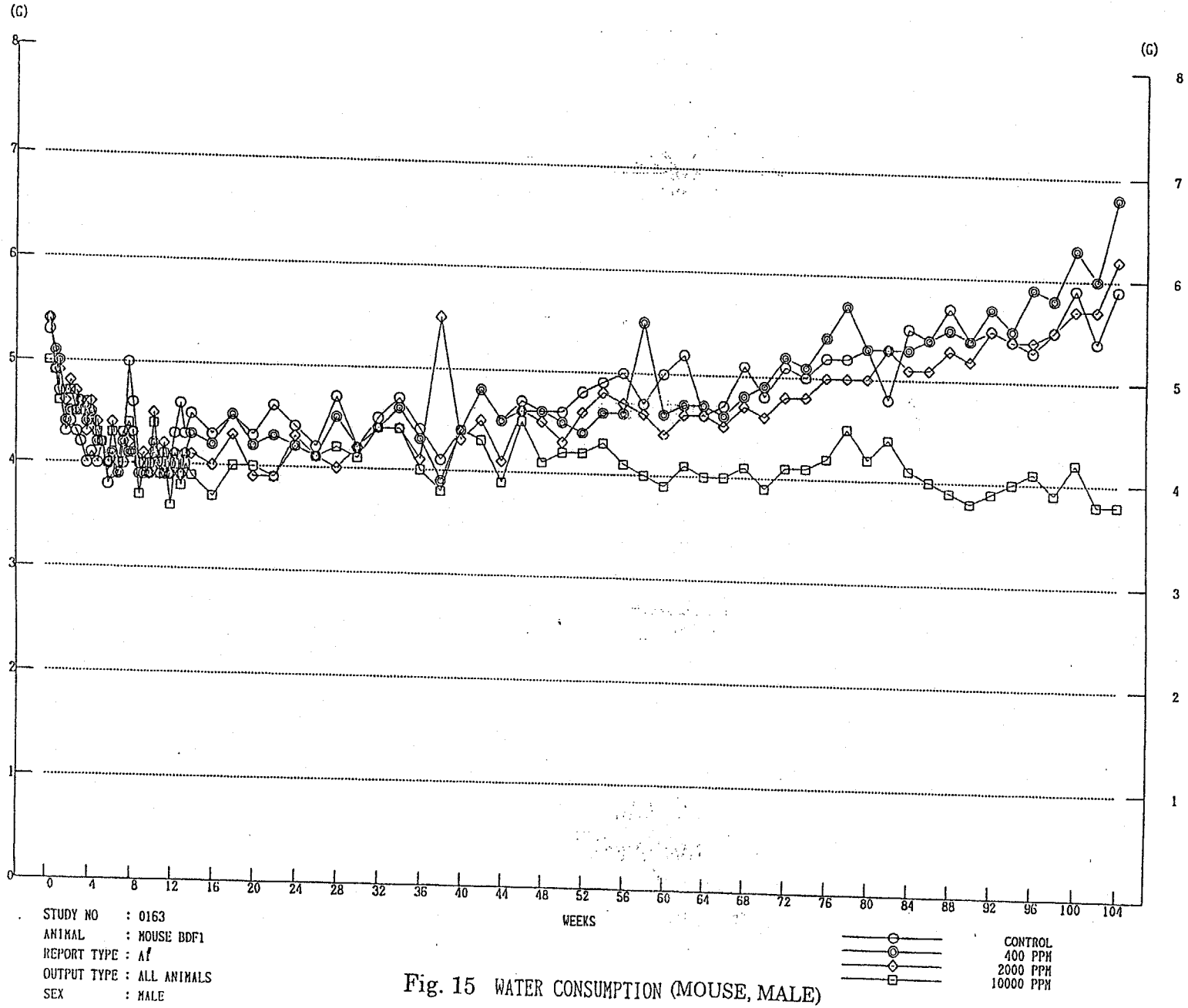


Fig. 15 WATER CONSUMPTION (MOUSE, MALE)

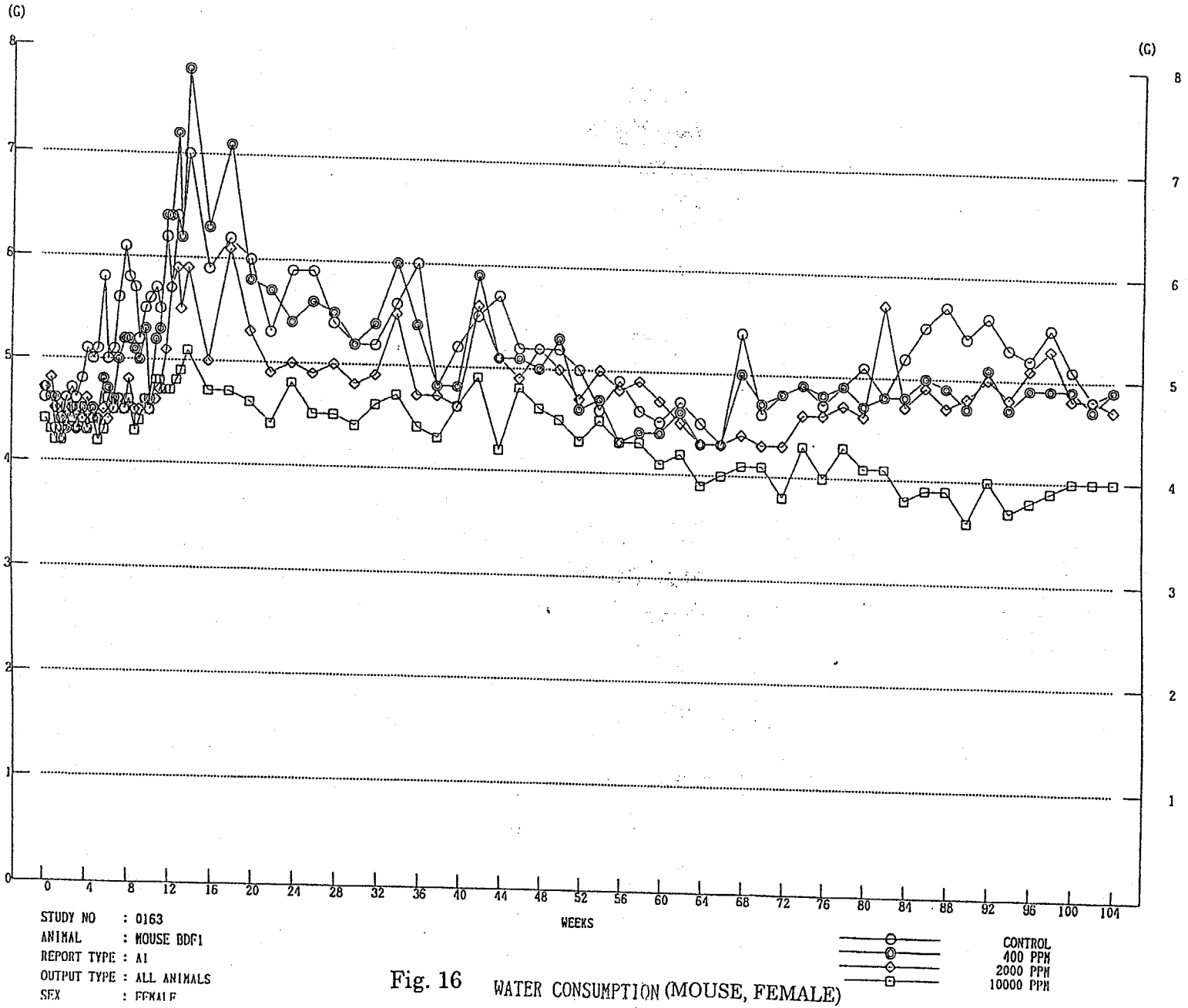


Fig. 16 WATER CONSUMPTION (MOUSE, FEMALE)